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Pre-operative quadriceps femoris neuromuscular electrical stimulation in total knee arthroplasty : a clinical and molecular analysis.

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Pre-operative Quadriceps Femoris Neuromuscular Electrical Stimulation in Total Knee Arthroplasty

A Clinical and Molecular Analysis

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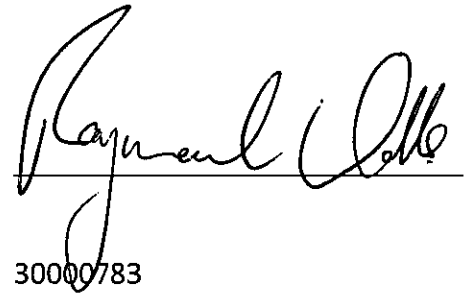
Declaration

I declare that this thesis, which I submit to RCSI for examination in consideration of the award of a higher degree (Doctor of Medicine), is my own personal effort. Where any of the content presented is the result of input or data from a related collaborative research program this is duly acknowledged in the text such that it is possible to ascertain how much of the work is my own. I have not already obtained a degree in RCSI or elsewhere on the basis of this work. Furthermore, I took reasonable care to ensure that the work is original, and, to the best of my knowledge, does not breach copyright law, and has not been taken from other sources except where such work has been cited and acknowledged within the text.

Signed: 

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The transition from clinical practice into research is daunting to say the least. The process of turning an initial idea into a feasible study requires much discussion, careful planning, and the acquisition of a new repertoire of skills. Without the guidance and support of Professor John O'Byrne this project would never have developed. His vision led the development of a clinically relevant study. Professor Niall Moyna has an enthusiasm for clinical research which is contagious. He was fundamental to the study design and implementation and contributed significantly to the analysis and editing of this thesis. The encouragement afforded to me from both Professor O'Byrne and Professor Moyna helped focus my efforts on the key study objectives. This project would not have been completed without them.

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Most of all, I am indebted to my closest friend and partner Heather and our son Cian. They have both made considerable sacrifices to enable the completion of the study and so I dedicate this thesis to them.

Pre-operative Quadriceps Femoris Neuromuscular Electrical Stimulation in Total Knee Arthroplasty: A Clinical and Molecular Analysis

Summary

Patients with knee osteoarthritis (OA) have asymmetrical muscle weakness due to neuromuscular activation deficits and muscle atrophy. Quadriceps muscle (QFM) strength declines after total knee arthroplasty (TKA) with associated functional impairment. The ultimate purpose of this investigation was to determine the effects of preoperative neuromuscular electrical stimulation (NMES) on quadriceps muscle strength and functional recovery after TKA.

Patients undergoing TKA for advanced knee OA were randomised into control or intervention (NMES) groups. NMES was applied to the affected QFM for 20 min, 5 days/week, for 8 weeks pre-TKA. QFM and hamstring (HS) strength were determined isokinetically and QFM cross-sectional area (CSA) calculated using MRI planimetry. Outcomes were assessed both objectively (walk, stair-climb and chair-rise tests) and subjectively (WOMAC, SF-36 and oxford knee scores). All evaluations were performed at baseline and preoperatively with strength and function also tested at 6 and 12 weeks post-TKA. Muscle samples were obtained from the vastus lateralis muscle at baseline and immediately preoperatively. Expression of myosin heavy chain (MHC) mRNA and genes associated with muscle hypertrophy (IGF-1) and atrophy (MAFbx and MURF-1) were determined using RT-PCR.

The NMES group increased isokinetic QFM strength (36%; p=0.008) and CSA (7.4%; p=0.036) preoperatively. Functional capacity also improved in the NMES group (walk, 9% [p=0.008]; stair-climb, 20% [p=0.008]; chair-rise, 34% [p=0.008]). MHC-IIx mRNA decreased by 42% indicating a fast to slow fibre shift. IGF-1 was upregulated in response to NMES, although MURF-1 and MAFbx did not change. Only the NMES group increased QFM strength from 6 to 12 weeks post-TKA (53%; p=0.011) with associated improvements in objective function. At 12

weeks post-TKA, the NMES groups were better than the control group at stair-climb (62%, p=0.029) and chair-rise (34%, p=0.019) tests. The control group had greater muscle atrophy than the NMES group at 12 weeks post-TKA (12.1% vs. 3.7%).

Substantial increases in preoperative muscle strength can be achieved following an unsupervised NMES program in subjects with advanced knee OA. Associated with this is an increase in muscle mass, IGF-1 expression and improvements in functional capacity. These effects translated into improved strength and functional recovery after TKA. We have also shown that changes in MHC isoform expression in response to NMES are similar to those seen with volitional exercise.

Introduction and Aims

Total knee arthroplasty is often necessary for the treatment of end-stage knee osteoarthritis when conservative measures have been exhausted. Subjects with advanced knee osteoarthritis have considerable muscle weakness adversely affecting function. After surgery muscle strength declines further which can take years, if ever, to fully recover.

We propose that a neuromuscular electrical stimulation (NMES) program will increase preoperative quadriceps strength with associated muscle hypertrophy and gains in functional capacity. The improvements in strength will translate into a more rapid post-operative functional recovery with relative preservation of muscle mass. At the molecular level, NMES training will induce down-regulation of genes implicated in muscle atrophy pathways (MuRF-1 and MAFbx) whereas IGF-1, involved in muscle hypertrophy, will be up-regulated. We predict that myosin heavy chain (MHC) type IIx expression will increase as more type II fibres are activated using NMES compared with volitional exercise. The principle aims of this study were to:

- Determine the efficacy of NMES in restoring quadriceps strength and function in subjects with advanced knee osteoarthritis.
- Determine changes in muscle CSA and gene expression in response to NMES.
- Determine if a preoperative NMES program can improve postoperative recovery in total knee arthroplasty.

Chapter I

Quadriceps Femoris Muscle Weakness – A Process of Aging and the Effects of Training

1.1 QUADRICEPS FEMORIS MUSCLE WEAKNESS

Muscle weakness markedly affects a person's ability to perform normal daily tasks. This in turn may negatively impact on their quality of life. Rowe and Khan separated aging into two processes – usual and successful.(1) Successful aging reflects a loss of function due to the effect of aging alone, whereas usual aging describes functional loss due to inactivity, disease and disability from conditions such as osteoarthritis. In this review, I will outline the principle mechanisms behind quadriceps femoris muscle weakness associated with successful aging and the detrimental effects on function as a consequence.

1.2 NEUROMUSCULAR ADAPTATIONS

Quadriceps weakness with increasing age has been attributed to several mechanisms culminating in an overall decrease in maximum force production. This strength decline can be attributed to morphological changes occurring within the muscle, adaptations at the level of the motor unit (MU), as well as by alterations in central and peripheral neural pathways.

1.2.1 Muscle Morphology

Sarcopenia is the principal muscular alteration responsible for the decline in strength with advancing age. This reduction in muscle mass results from a combination of muscle fibre loss and individual fibre atrophy.(2-6) Changes can be seen as a reduction in whole muscle cross-sectional area (CSA) performed using various radiological imaging modalities or by analysis of muscle specimens obtained percutaneously.(7-18) When stained by ATPase histochemistry, it is possible to differentiate the fibres into type I (slow twitch) or types IIa and IIb (fast twitch) to determine fibre composition and distribution (Figure 1.1).(19)

Imaging modalities may not reflect effective muscle tissue mass since older people have a greater proportion of non-contractile tissue (fat and connective tissue) which negatively affects force production.(5;13;14;20;21) There is a two to three fold increase in non-contractile components in older adults which is associated with a decreased activity level.(12;14) Despite this limitation, radiological assessments are more accurate that simple clinical anthropometric methods which typically under or over-estimate muscle mass, especially in obese subjects.(7;22)

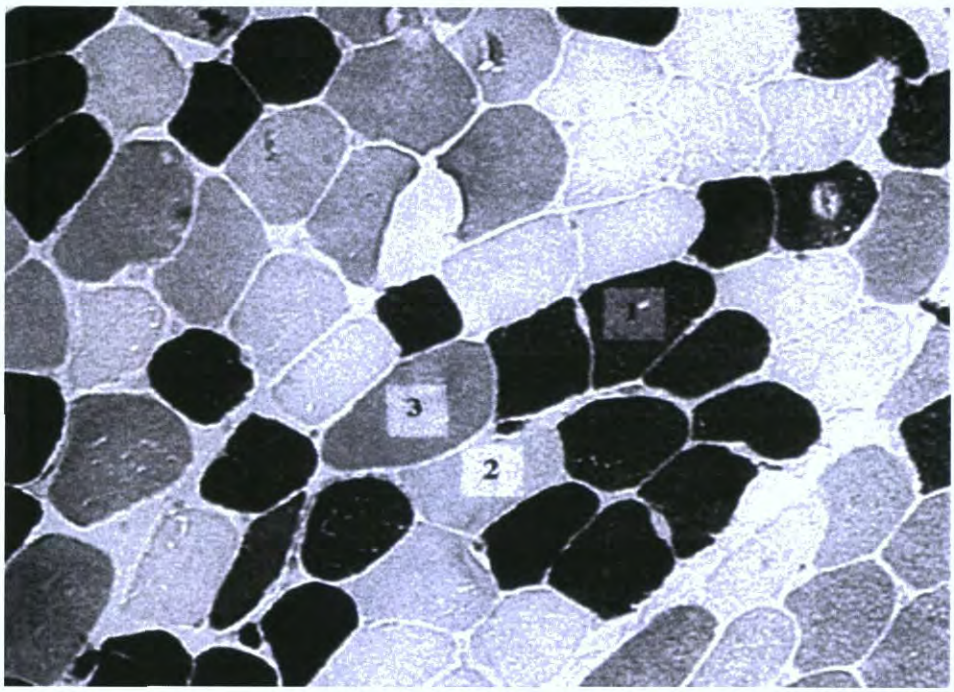


Figure 1.1. Histochemical staining of a muscle specimen. Fibres designated 1, 2, and 3 represent types I, IIa, and IIb respectively. (Williamson et al, J Appl Physiol 2000;88:627-633)

Although the process of sarcopenia starts in young adults (3rd decade) it accelerates in the 5th decade producing declines of 24% to 40% in muscle cross-sectional area between the ages of 20 and 80 years.(5;9;23;24) Such reductions in mass are associated with weakness and increased morbidity in older adults,.(15;25;26) Muscle atrophy occurs at a rate of 1.0-1.4% per year from 60 years of age, whereas annual strength declines range from 1.5-3%.(6;11;12;27) Since muscle atrophy only explains about 50% of strength loss, additional mechanisms must

play a role in potentiating quadriceps muscle weakness in otherwise healthy individuals. These include contractile properties, motor unit function and neurological control.(12;20;28)

1.2.1.1 Muscle Fibre Number

It has been suggested that a reduction in the total number of muscle fibres affects muscle mass to a greater extent than individual fibre atrophy.(6;29) While there is agreement that the number of fibres decreases with advancing age, disagreement remains as to the proportionate changes of type I and type II fibres. Several studies have found no change in the proportion of type I and II fibres , including the subgroups IIa and IIb.(6;27;30) In contrast, Frontera et al found a decrease in the number of type I fibres whereas Larsson et al reported a selective loss of type II fibres.(11;31)

Most of the studies are confounded by the fact that they analysed only small numbers of muscle specimens from relatively heterogeneous cohorts and consequently must be interpreted with caution. Furthermore, we now know that muscle fibres of elderly subjects often coexpress more than one myosin heavy chain (MHC) isoform used for histochemical staining and fibre type determination.(19;32;33) This could result in the incorrect interpretation of individual fibre types during analysis.

1.2.1.2 Muscle Fibre Size

Changes in muscle fibre size associated with increasing age affects fibre types differently. Type I fibre CSA does not change significantly whereas type II fibres are preferentially reduced in size.(3;5;6;11;29;31;34-36) Such changes are more pronounced sedentary individuals.(24) Specifically, type II fibres are up to 20% larger than type I fibres in subjects under 40 years of age, but by the 8th decade this is reversed with the CSA of type II fibres up to 50% less than type I fibres.(37;38) Since there is also a strong relation between type II fibre atrophy and strength decline it is likely that type II fibres play a smaller role in force generation in older adults.(20;39)

1.2.1.3 Contractile Properties

A reduction in contractile properties further potentiates the decrease in force generating capacity of skeletal muscle.(2;3) The maximal force generated per unit CSA of a muscle fibre is known as specific tension and relates to the intrinsic force potential of muscle. Both contractile velocity and specific tension are reduced in sedentary and physically elderly individuals compared with young subjects.(5;25;36;40) Petrella et al found that the decline was more marked in females and suggested an inability to quickly contract the quadriceps muscle for antigravity effects may contribute to the risk of falls.(25) Myosin concentrations in individual fibres also reduces with age and correlates directly with specific force, possibly due to a reduction in actin-myosin interactions.(33)

1.2.2 Motor Units

Maximal force production requires recruitment of all motor units (MU's) firing at their maximum discharge frequency. With aging, there is a reduction in the overall numbers of motor units complexes.(5;28;41) This process begins in the third decade with accelerated loss over 60 years of age.(5;28) Individual MU size increases due to surviving motor neurones reinnervating denervated fibres through collateral sprouting, producing an increased innervation ratio.(2;3;5;28)

1.2.2.1 Motor Unit Remodelling

The concept of remodelling explains the normal turnover that takes place at the neuromuscular junction.(5;42) This cyclical process involves denervation of a muscle fibre with subsequent neuronal axon sprouting to reinnervate the muscle. In older adults, this process is altered so that parent MU's associated with type I fibres reinnervate collateral type II fibres which appear to be selectively denervated.(5) The underlying aetiology and mechanisms remain unclear. It has been suggested that there is faster axonal growth from neurons innervating type I fibres which also form better MU connections than neurons supplying type II

fibres.(5) The concept of remodelling helps explain the variability of MU firing rates as well as the increased co-expression of myosin heavy chain isoforms in muscle fibres of older people.(3;19;32;43;44)

1.2.2.2 Motor Unit Discharge Frequency

The action potential of a single MU can be recorded using an electrode consisting of 2 fine wires inserted into the muscle belly. Although it cannot confirm if all MU's have been recruited a submaximal discharge rate reflects incomplete muscle activation.(45) No significant decline has been observed in the quadriceps muscle with increasing age.(40;41) This supports studies that found no change in fibre type proportions as reduced firing rates would be associated with a reduction in the numbers of fast twitch fibres. The number of MU's can also be estimated using this technique and a reduction has been associated with decreased torque generation in elderly individuals.(46)

1.2.3 Neural Signalling and Voluntary Activation

Voluntary muscle activation is defined as the level of neural drive to a muscle during maximal voluntary contraction.(2) It involves signal transmission from both cortical neurons (cortico-spinal pathways) and motor neurons at the spinal cord level.

A variety of methods have been described to evaluate neuromuscular activation. The interpolated torque technique (ITT) involves the application of supramaximal electrical stimulus to the motor axons during a maximal voluntary isometric contraction (MVIC) with the aim of recruiting fibres which have not been voluntary activated thereby producing additional torque.(47) Voluntary activation using the ITT is defined as the ratio of the superimposed torque during MVIC to the evoked torque measured at rest (IT ratio).(2;48) The central activation ratio (CAR) is a similar stimulation method, considered as the proportion of overall torque that is produced voluntarily.

Behm et al assessed quadriceps femoris activation in young healthy men and found the ITT more accurate for estimating activation failure than the CAR.(49) Surface electromyogram (EMG) recording is a non-invasive method that records electrical activity of motor units (MU's) using recording electrodes secured to the overlying skin. As it does not differentiate between central and peripheral afferents it may underestimate activation levels, and is also influenced by the level of subcutaneous fat which decreases the received signal.(2)

1.2.3.1 Voluntary Muscle Activation in Elderly Subjects

It is still unclear if a deficit in voluntary muscle activation occurs with increasing age. Stackhouse et al found reduced levels of activation in subjects aged over 65 years compared with a younger cohort.(50) Similarly, Stevens et al found that elderly subjects with a mean age of 73yrs have an average deficit of 4.5% compared to only 1.9% in young subjects (mean age, 24yrs).(51) Both of these studies assessed activation using the CAR technique. Conversely, Roos et al, found similar activation deficits of approximately 6% in young and elderly subjects using the ITT.(40) Since all three studies reported much greater strength deficits than activation deficits (40% versus 4-6%) in their elderly subjects, voluntary activation failure does not explain the significant loss in quadriceps strength with age. (40;50;51)

Assessment of activation using these stimulation methods assumes the relation between muscle force and voluntary activation is linear. However it has recently been shown to be curvilinear.(4;52) Hence, small activation deficits would correspond with greater reductions in torque generation. When the studies by Stackhouse et al and Stevens et al were reanalysed and corrected to assume a curvilinear relationship, the difference increased substantially with elderly subjects exhibiting significantly greater voluntary activation deficits than the younger cohort (13% vs 2%).(52) Harridge et al found activation deficits ranging from 7% to 31% using the ITT in subjects over 85 years of age.(48) It has been suggested that the large deficits may be due to the fact that this cohort was less physically active than the healthy elderly subjects reported in other studies.(2) Nonetheless, it is clear that voluntary muscle

activation decreases with advancing age, substantially contributing to quadriceps muscle weakness.

1.2.3.2 Muscle Coactivation

The hamstring muscle group is considered the antagonist to the quadriceps femoris muscle. While a degree of antagonist coactivation is necessary to assist in joint stability and limit excessive tibial internal rotation near full knee extension, an imbalance results in impaired quadriceps effectiveness.(53;54) Coactivation is determined by simultaneous EMG analysis during maximal voluntary contractions where the antagonist action is expressed as a percentage of its activity when acting as an agonist.(53;55) Increased antagonist coactivation reduces agonist force production by direct opposition and reciprocal inhibition.(56;57) In elderly subjects there is increased antagonist coactivation during both isometric and dynamic concentric contractions which adversely affects quadriceps muscle strength.(58;59) In summary, central nervous control adversely affects force production in elderly individuals due to impaired agonist activation and/or increased antagonist coactivation.

1.2.4 Myosin Heavy Chain Composition

Muscle fibres are comprised of dozens of myofibrils which themselves are formed from repeated elements known as sarcomeres, the functional unit of muscle. A sarcomere contains actin and myosin myofibrillar proteins and in turn, each myosin filament is composed of several hundred myosin molecules. The myosin in skeletal muscle consists of two heavy chains and four light chains which form a molecule with two globular heads and a long tail (Figure 1.2).(60) Myosin heavy chain (MHC) is the principle contractile protein which determines the rate of force development (contractile speed). There are three isoforms expressed in human skeletal muscle fibres: MHC I (slow), MHC IIa (fast), and MHC IIx (fastest). Myofibrils are classified as type I, IIa or IIb based on the predominate MHC isoform expressed.

Muscle fibre composition is normally determined by myosin ATPAse staining histochemistry. Although this method can detect type I, IIa, and IIx fibres, hybrid fibres that coexpress two or more *isoforms* may not be accurately identified.(19) Combining protein gel electrophoresis (SDS-PAGE) with silver staining permits the classification of myosin heavy chain (MHC) main (I, IIa, IIx) and hybrid (I/IIa, I/IIa/IIx, IIa/IIx) isoforms.(19;24;32;36;61) It should be noted that many researchers report MHC IIb and/or MHC IIx. This is because the human isoform (MHC IIb) contains similar transcript homologues to the rat isoform (MHC IIx) and the two are considered interchangeable.(19)

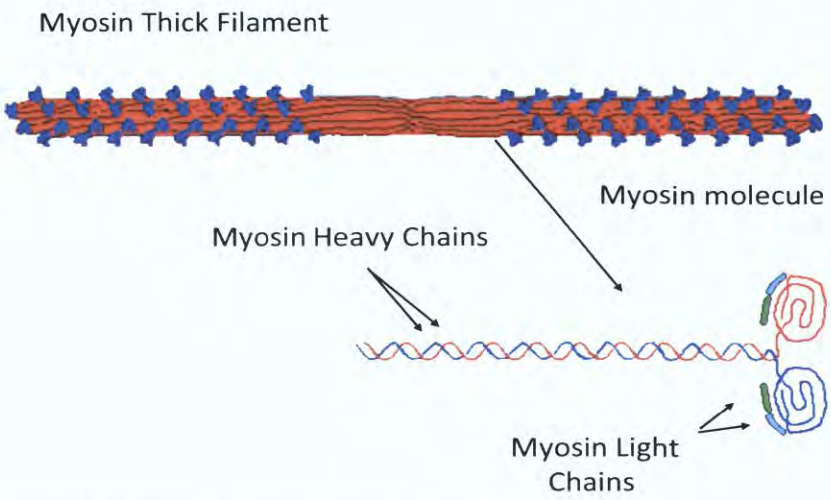


Figure 1.2. Structure of thick filaments and myosin molecules

MHC isoform synthesis decreases with increasing age.(62) There is evidence of increased MHC isoform coexpression in single muscle fibres suggesting transition towards a faster fibre type.(32;44) The most frequent hybrid fibre found is type I/IIa, although in immobilised subjects the type IIa/IIx hybrid is dominant.(33;44) There is also evidence of fibres switching from one type to another along their lengths.(63) Not all studies have found evidence of a slow to fast shift with increasing age. Larsson et al reported that sedentary elderly men had higher quantities of MHC type I while active elderly men had similar levels to young subjects.(36) However they only examined muscle specimens from four elderly men and two young men. Some studies have only examined overall proportions of MHC isoforms in

muscle samples without analysing fibre phenotype. Short et al found an increase in MHC type I with an associated decrease of type IIa and IIx with advancing age suggesting a fast to slow phenotype shift.(18) *In contrast*, Puhke et al found no change in the proportion of MHC isoforms with age.(64) Certainly it is possible that increased MHC co-expression with an increase in type I/IIa hybrids may still have occurred in both studies.

The increase in co-expression of MHC isoforms with advancing age can result from a number of factors The reinnervation of type II fibres with neural afferents from type I neurons may produce mixed cell signalling with resultant MHC isoform coexpression. Another explanation is that alterations in protein synthesis, possibly due to the upregulation of muscle regulatory genes such as myogenin, may attempt to differentiate the muscle cell into a different type.(44) There is also a concept of fibre type transition towards a stimulus or stability.(19) Disuse has been shown to produce a transformation from slow to fast fibres. However, it has been suggested that MU remodelling causing denervation of type II fibres may offset this change.(33) An interplay between these mechanisms, that are influenced by physiological, pathological and activity levels, may explain why some studies failed to find evidence of a slow to fast shift in fibre type.

Early research did not find a relation between muscle strength and changes in the proportion of MHC isoforms expressed.(12;24;28) Short et al found an inverse relation between muscle strength and MHC-I expression, and a direct relation with types IIa and IIx. (18) However no associations were found when adjusted for muscle CSA. More recent work has shown that myosin concentration in a fibre is related to its specific force and both are reduced with increasing age.(33)

Few studies have investigated changes in pre-translational MHC gene expression (messenger RNA [mRNA]). Some have found no difference in levels of MHC mRNA expression when comparing young and old subjects.(65-67) In studies that reported age differences, MHC mRNA decreased for types IIa and IIx while type I mRNA was either increased or unchanged in

older adults.(18;68) These findings support those who reported preferential type II fibre atrophy on CSA analysis. A decrease of type II mRNA transcripts may be due to changes in the rate of gene expression with increasing age or alternatively altered stability of mRNA. Some fibres may express one isoform at the protein level (eg. MHC IIx) but another at the genetic level (eg. IIa) indicating incomplete fibre transition.(69)

1.2.5 Molecular Mechanisms of Skeletal Muscle Mass

Muscle mass is maintained by a fine balance between protein synthesis and degradation pathways. Muscle wasting occurs when synthesis is decreased or breakdown is increased, a process which is promoted by disuse and immobilisation.(60) In contrast, hypertrophy occurs in response to mechanical overload such as resistance training. The molecular mechanisms associated with sarcopenia are only beginning to be elucidated. Both anabolic and catabolic pathways have been implicated with a chronic imbalance favouring catabolism resulting in muscle wasting.(70) The principal hypertrophy and atrophy signalling pathways currently understood are outlined in figure 1.3.

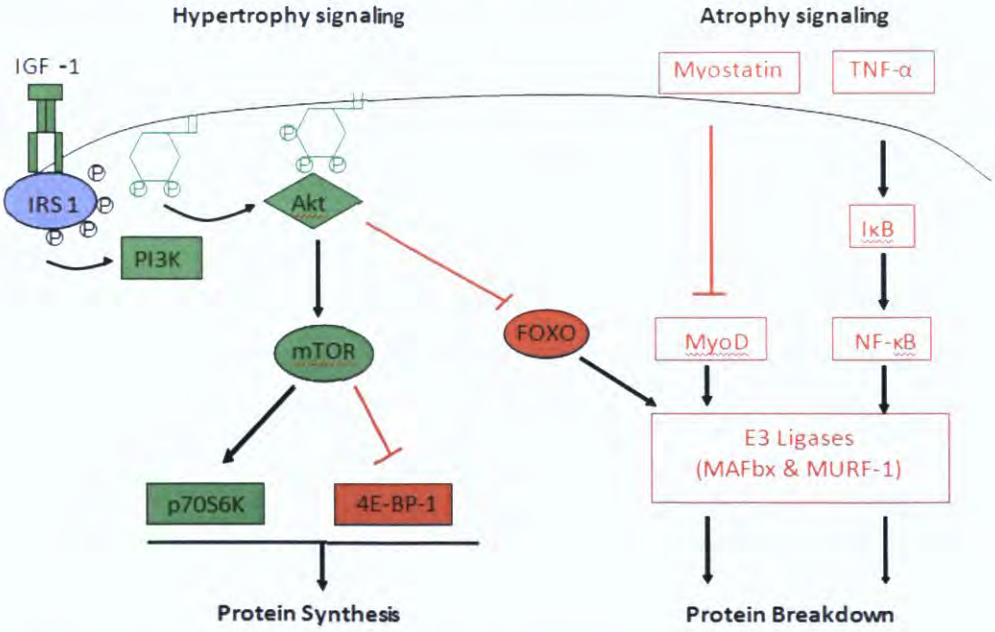


Figure 1.3. Hypertrophy and atrophy signalling pathways

1.2.5.1 Muscle Atrophy Pathways

The major catabolic pathway resulting in myofibrillar degradation is the Ubiquitin-Proteasome Pathway (UPP) which, in normal conditions, helps regulate cell turnover.(70) Proteasomes are large protein complexes that degrade damaged or unneeded proteins through proteolysis and are activated by covalent attachment of ubiquitin. The process requires three distinct enzymatic components, an E1 ubiquitin activating enzyme that adenylates the ubiquitin molecule, an E2 conjugating enzyme and an E3 ubiquitin ligating enzyme that recognizes the target protein and catalyzes the transfer of ubiquitin.(70) The E3 ubiquitin ligases determine substrate specificity.

Although several hundred E3 enzymes have been identified, screening studies in multiple models of skeletal muscle atrophy have identified two genes known to encode E3 ligases that have a significant role in skeletal muscle atrophy. These are muscle atrophy F-box (MAFbx, also known as Atrogin 1) and muscle-specific RING Finger-1 (MURF-1).(71) Bodine et al have shown that MAFbx and MURF-1 knockout mice (encoding genes removed) had less gastrocnemius muscle atrophy compared to controls 14 days after sciatic nerve denervation (56% and 36% respectively).(71) Likewise, using arthritis rats, Granado et al found that muscle atrophy was related to the upregulation of both MAFbx and MURF-1.(72) Recently, Jones et al showed that both MAFbx and MURF-1 were also upregulated in human muscle following two weeks of lower limb immobilization.(73) Similarly, Ogawa et al found increased expression of MAFbx in association with a 4% reduction in muscle CSA following 20 days bed-rest.(74) Accordingly, Welle et al observed higher levels of mRNA encoding components of the UPP in elderly subjects compared to healthy young subjects.(65)

In addition to the UPP, additional proteolytic enzymes including caspases, lysosomal enzymes (cathepsins) and calpains have been found to play a role in muscle catabolism.(70;75;76) It appears that they assist in the early stages of myofibrillar degradation

breaking down actomyosin complexes so that subsequent ubiquitination can occur. Insulin and IGF-1 attenuate the effects of atrophy by inhibiting caspase-3.(75)

Nuclear Factor Kappa B (NF-κB) is classically activated by tumour necrosis factor α (TNF-α). TNF-α induces intracellular release of NF-κB from inhibitory proteins (IκB) which then translocates into the cell nucleus promoting transcription of UPP components.(77) Although TNF-α is generally released in inflammatory conditions, NF-κB is also activated through non-classical pathways *in disuse* atrophy.(78) A NF-κB knockout mice study demonstrated resistance to atrophy of both type I and II fibres following 10 days of immobilisation. NF-κB is also implicated in the MHC shift from slow to fast isoform expression.(79) The Forkhead Box, Class O (FOXO) group of gene transcription factors upregulates components of the UPP. In particular FOXO 3 upregulates MAFbx.(80) FOXO is linked to the main hypertrophy pathway (IGF/Aky/PI3K) via Akt which has an inhibitory effect. Accordingly, an increase in IGF-1 blocks FOXO transcription factors through phosphorylation and hence suppresses MURF-1 and MAFbx.(75;81)

Myostatin, a member of the transforming growth factor-β (TGF-β) family, is produced by myocytes and exerts autocrine and paracrine effects. It is a negative regulator of muscle mass that acts by inhibiting the synthesis and activity of myogenic differentiation factor D (MyoD), an essential transcriptional regulator of the components of catabolic pathways.(70)

Sarcopenia and disuse atrophy are associated with up to a thirty-fold upregulation in myostatin expression.(82;83) In subjects with disuse as a result of hip osteoarthritis, higher levels of myostatin were associated with smaller type II fibre CSA.(83) This is not a universal finding however with Welle et all reporting no difference in myostatin expression with increasing age.(84) Conversely, Lin et al found a two-fold increase in muscle mass in myostatin knockout mice.(85) However Amthor et al found that this muscle was intrinsically weaker producing a lower specific force.(86) The lack of force production may be explained by a greater proportion of type II fibres which are more fatigable as well as a slow to fast shift in

MHC isoform expression.(87) Glucocorticoids exert various catabolic effects of which one is the upregulation of myostatin.(88)

1.2.5.2 Muscle Hypertrophy Pathways

The principal anabolic pathway is the Insulin-like Growth Factor-1 (IGF-1)/Phosphatidylinositol 3-Kinase (PI3K)/Akt pathway (Figure 1.3).(70) The process is initiated by IGF-1 binding to its cell membrane receptor which activates insulin receptor substrate-1 (IRS-1). IRS-1 activates PI3K which in turn phosphorylates and activates Akt. Subsequent activation of the mammalian target of rapamycin (mTOR) results in increased protein synthesis by activating p70S6 kinase (p70S6K) and inhibiting 4E binding protein 1 (4E-BP-1). P70S6 K is a serine / threonine kinase that phosphorylates the S6 ribosomal protein inducing protein synthesis. 4E-BP-1 is a negative regulator of the protein initiation factor eIF-4E, and so its phosphorylation via mTOR relieves inhibition of protein synthesis. In vivo studies have shown upregulation of Akt/mTOR during muscle hypertrophy and downregulation in atrophy.(Bodine sc 2001) In addition, the normal hypertrophic response of the plantaris muscle in mice following removal of the synergistic gastrosoleus compex is blocked in the presence of rapamycin due to inhibition of mTOR.(89)

IGF-1 is a polypeptide with high sequence similarity to insulin. It is released systemically from the liver in response to systemic growth hormone (GH) and locally from myocytes and acts in an autocrine and paracrine manner. Local IGF-1 may have a greater role in regulating muscle mass.(60) DeVol et al found that rats who had their anterior pituitary gland removed had a 13-fold increase in IGF-1 mRNA in response to induced muscle hypertrophy.(90) There is an elevation in plasma IGF-1 concentrations in elderly male subjects following subcutaneous injection of GH but no effect is found in levels of muscle IGF-1 mRNA indicating that local factors are dominant in muscle mass homeostasis.(91) Six IGF binding proteins (IGFBP's) modulate either positively or negatively the bioavailability of IGF.(70) GH also affects IGFBP levels, further modulating the effects of IGF-1. Of these binding proteins,

IGFBP-5 is most important in the regulation of myocytes. It binds to and inactivates IGF-I and is also thought to act as an independent bioactive peptide. Salih et al found that over expression of IGFBP-5 leads to a 31% drop in skeletal muscle in mice.(92)

Muscle atrophy occurs when IGF-1 levels are insufficient.(75) With increasing age, Welle et al found that muscle IGF-1 expression declined although the functional importance of this was unclear since their levels did not correlate with muscle mass or myofibrillar protein synthesis rates.(84) Reardon et al also observed upregulation of IGF-1 mRNA by up to 3-fold in subjects with disuse secondary to hip osteoarthritis.(83) The authors suggested the increase was a counter-regulatory response to muscle atrophy. In relation to the down-stream targets, detraining results in decreased activation of Akt and an increase in FOXO content.(93)

1.3 QUADRICEPS WEAKNESS AND FUNCTIONAL CAPACITY

1.3.1 Muscle Strength

There is significant muscle weakness associated with aging. Absolute strength is determined by isometric assessment, whereas isokinetic testing provides a better reflection of functional impairment with evaluation of both concentric and eccentric actions. The decline in quadriceps muscle strength with age is curvilinear. After peaking in the third decade, isometric and concentric isokinetic quadriceps peak torque declines in the fourth decade with accelerated loss in the seventh decade so that subjects in their 70's and 80's have up to a 55% reduction compared to younger adults.(5;9;10;12;20;21;94-96)

There is limited data on the effects of aging on dynamic eccentric muscle strength. Several reports with limited sample numbers have provided evidence that eccentric strength declines to a lesser extent than concentric strength.(35;97-100) One explanation is that connective tissue prevents stretching during eccentric motion and an increase in connective tissue with age could attenuate the rate of decline in eccentric strength.(96;97) Conversely, Lindle et al analysed a cohort of 654 subjects aged between 20 and 93 years and found that

both eccentric and concentric strength declined at a similar rate.(96) While concentric strength decreased in men and women from the fourth decade, declines in eccentric strength in women did not commence until the fifth decade.(96) Cumulative data from available literature on quadriceps strength decline with aging is presented in table 1.1.

Table 1.1. Average declines in quadriceps femoris peak torque in subjects over 60 years compared to subjects under 45 years from current literature.

Assessment Modality	Decline in Peak Torque (%)
Isometric:(10;15;21;24;40;95)	32.0-51.0%
Isokinetic Concentric:(15;20;26;96;98-100)	
Low angular velocity (≤90 degrees/sec)	17.6-53.4%
High angular velocity (≥120 degrees/sec)	23.0-34.1%
Isokinetic Eccentric:(35;96;98-100)	
Low angular velocity (≤90 degrees/sec)	10.0-38.3%

Specific force is the force generated per unit muscle area (force/CSA) and should remain constant for strength decline to be explained by muscle atrophy alone.(12;13;15) While specific force remains somewhat constant in younger people, it decreases by approximately 1.5% per year after 65 years of age, due to the compounding effects of neural and motor unit adaptation.(12) Such age related declines in specific force have been well established in the literature.(10;13;15;24;25;94;96)

1.3.2 Sustained power and fatigue

Although peak torque gives a measurement of absolute muscle power, it is maintained for only a brief period. Assessment of sustained or critical muscle power allows evaluation of functional capacity as it is influenced by metabolic factors affecting oxidative and anerobic capacities which also decline with age. Critical power (CP) is considered as the maximal rate of non fatiguing work when a physiological steady state has been reached. When tested using a 24 minute cycle ride, healthy elderly male subjects had a 35% reduction in CP compared to younger subjects.(101)

Muscle fatigue is the decline in force generating capacity of muscle during repeated or sustained contractions. Many daily activities such as walking and stair climbing require repeated quadriceps contractions as opposed to achieving maximal torque. Katsiaras et al assessed the fatigability of the quadriceps muscle by performing fast isokinetic repetitions in 1512 elderly men and women.(13) Weaker subjects were found to be more fatigue resistant. (13) Dynamometry is considered an open-chain activity although fatigue and strength can also be assessed using closed chain methods (e.g.-sit to stand).(25) An age related decline has been found with "open-chain" knee extension tests but not in a "closed-chain" sit to stand test or in sustained isometric contractions.(5;25) Familiarisation of a technique may provide some explanation for this discrepancy. A reduction in oxidative enzymatic activity may also describe why fatigability does not significantly change with aging despite the greater proportion of "fatigue resistant" type I fibres.(5)

1.3.3 Performance in Activities of Daily Living

Adequate quadriceps muscle strength is necessary for the performance of tasks such as walking, climbing stairs and rising from a chair, as well as assisting in joint stability. Muscle weakness has been associated with a greater risk of falling in the elderly.(102;103) Older subjects with quadriceps weakness require significantly greater physiological effort when rising from a chair suggesting diminished reserve capacity. (25;104-107) During voluntary muscle contraction type I fibres are recruited first followed by type II fibres. Since chair-rising and stair-climbing require the production of relatively high forces, the preferential atrophy of type II fibres with aging helps explain this functional deficit. Hip, abdominal and lower leg muscle strength are contributory factors when rising from a chair, and are also weakened with age.

A decline in walking speed is associated with quadriceps muscle weakness.(105;108;109) Brown et al, found a stronger correlation between strength and walking speed in individuals aged between 60-72 years compared with even older adults (75-88 years).(104) The relation between strength and gait speed is curvilinear such that small

changes in physiological capacity substantially effect performance in frail adults, whereas large changes may have little impact on the performance of tasks in young healthy adults.(110) Rantanen et al have examined the non-linear relationship between quadriceps strength and gait speed using regression analysis.(111) They found that 2.3 Nm/kg (knee extension peak torque/BMI) was the threshold beyond which an increase in strength did not produce an increase in maximum gait speed. Although additional factors play a role in normal function, such as balance, joint pain and disease, as well as the interplay of other joints, it is clear that quadriceps muscle weakness contributes significantly.

1.4 QUADRICEPS STRENGTHENING PROGRAMS

1.4.1 *Training* Programs

Endurance training improves stamina and aerobic capacity whereas resistance training increases muscle force. Resistance training can be further divided into progressive (PRT) or high intensity programs. With graduated programs the training intensity is progressively increased over several weeks.(3) High intensity programs encourage subject to perform high intensity, low repetition programs for 3 days per week.(3) Resistance training programs can be altered by manipulating the type, intensity, frequency and duration of exercise.(3) Typical exercises include knee extension, leg press and squats.(112;113)

1.4.2 Resistance Training

Resistance training has been shown to increase quadriceps strength in the elderly. Isokinetic quadriceps and hamstring strength increased by 10-17% and 15-19% respectively in men aged between 60 and 72 years following 12 weeks high intensity training.(114) In addition quadriceps CSA increased by an average of 9%. Hakkinen et al studied the effect of combining resistance training and explosive strength training (rapid contractions with lighter loads) in a similar aged cohort over 6 months.(115) Strength improved by 36-57% and muscle

CSA by 6%. The improvements were generally greater in women. However, others have found greater quadriceps muscle hypertrophy in men than women, independent of age,.(116;117)

Increases in quadriceps muscle strength are similar in young and older subjects following progressive resistance training (PRT).(112;113) Petrella et al found a 17-29% increase in strength in the first 8 weeks of a 16 week PRT program (17-29%) compared with 9% in the final 8 weeks.(112) Ten weeks PRT increased isometric peak torque by 16% in both young and older men.(113) Quadriceps CSA increased by 10% with additional neural adaptations potentially explaining the greater changes in strength.(113)

Resistance training can produce significant Improvement in quadriceps strength in very frail subjects up to 96 years of age.(108) An 8 week high intensity program increased quadriceps strength by 174% and CSA by 9%.(108) Harridge et al found increases in quadriceps strength in 14 subjects aged between 85-97 years following a 12 week PRT program.(48) There were marked differences in quadriceps strength gain determined by isometric evaluation and by the maximum amount of weight that subjects could lift (1-RM; 1 repetition maximum). While 1-RM improved by 134%, isometric strength increases were only 37% at 60° knee flexion and 17% at 90° knee flexion.(48) Training programs typically require subjects to perform weightlifting tasks and the skills acquired may contribute to the more dramatic increases seen when strength is determined as changes in 1-RM. Dynametric evaluations are performed only intermittently with lower skill acquisitions reflected by smaller changes.(48) Harridge et al also compared changes in gross and lean muscle (excluding fat and connective tissue) CSA.(48) Gross CSA increased by 4.8% whereas lean CSA increased by 9.8%. There was considerable variability with increases in lean CSA ranging from 2% to 44%.(48)

1.4.3 Long-term Training

Longitudinal studies have shown that men and women aged over 70 years who maintain their activity levels experience less declines in quadriceps strength decline compared to sedentary subjects.(118;119) Elderly subjects who participate in long term strength-training

programs can also have similar quadriceps strength and CSA compared to younger individuals.(24) Such maintenance of strength is not found in elderly subjects who participate in regular but lower intensity activities such as swimming or running.(24) Hence, physical activity may only prevent a decline in quadriceps strength if performed at sufficient intensity levels.

1.5 NEUROMUSCULAR ADAPTATION FOLLOWING STRENGTH TRAINING

Older subjects can increase muscle strength and mass following resistance training. Since gains in strength are greater than muscle mass, nonhypertrophic adaptation must concomitantly occur.

1.5.1 Muscle Fibre Adaptation

There is consensus that muscle fibre hypertrophy occurs following strength training. A 12 week high intensity training study found a 34% and 28% increase in type I and II fibre CSA respectively in men over 60 years of age.(114) Hakkinen et al also found an increase in the size of type I and IIa in young and old men after 10 weeks PRT.(113) Although endurance training increases muscle fibre size, it is to a much less degree than resistance training.(120) Kosek et al reported evidence of differential myofibre hypertrophy with age in response to 16 weeks resistance training.(121) Increases in type I fibre CSA were only found in the young cohort.(18%). Although both young and older subjects increased type II CSA, mainly due to type IIa hypertrophy, it was again more marked in the young subjects (32% vs. 23%).(121) Such differences may be due to impaired regeneration processes in older subjects.

Some studies have found no significant change in fibre type distribution following PRT.(32;114) Sipila et al reported an increased proportion of type I fibres after 18 weeks intensive strength training in elderly women.(120) Several studies have found no change in the amount of type I fibres although did reported changes in type II fibre composition.(113;121) In particular, a decrease in the numbers of type IIx fibres was

associated with increases of type IIa and IIax hybrid fibres indicating a fast to slow fibre shift.(113;121)

1.5.2 Motor Unit Adaptation

Surface EMG has also shown a reduction in MU activation after 8 weeks of PRT in older subjects (60-75 years) with more substantial improvements occurring in women.(112) This indicates that performance of a specific task requires less demand physiologically after training and may be due to improved MU coordination or efficiency. Increased EMG activity during maximal muscle contractions could be due to an increased MU firing rates, or an increase in the number of active MU (recruitment).(48;115) A recent review concluded that training can increase MU firing rates as well as the rate of force development.(122) Little is currently known about the effects of higher neuronal control in response to training and the individual contributions they play in MU activity.

1.5.3 Neural Adaptation

Moritani suggested that neural factors were the principal contributing factor to early strength gains following resistance training in the elderly.(123) This is supported by recent work where quadriceps isometric strength increased by 13-16% following only 2 weeks of resistance training in young men.(124) MRI analysis demonstrated a contrast shift without hypertrophy indicating increased muscle contractility.(124)

Neural activation, measured as surface EMG activity during maximal isometric contractions, has been shown to significantly increase in young and older subjects following strength training.(112;113;115;125) This indicates decreased neural inhibition. Petrella et al found that neural adaptations are responsible for the changes in muscle strength within 8 weeks of training while changes in muscle CSA are responsible for later gains.(112) Only Harridge et al failed to find an improvement in muscle activation following 12 weeks PRT in subjects aged over 85 years.(48)

Carolan and Cafarelli found a reduction in hamstring coactivation in young men after 2 weeks of isometric resistance training.(56) These changes have been replicated in middle-aged and elderly subjects after 6 months of resistance training.(115) A reduction in biceps femoris activation was observed using surface EMG during maximal knee extension. It is unclear if such changes reflect adaptation in central or peripheral neural control.

1.5.4 Myosin Heavy Chain Composition

Williamson et al studied the effects of 12 weeks PRT on 7 elderly male subjects (mean age, 74 years).(19) The numbers of hybrid fibres coexpressing 2 or more MHC isoforms decreased by 22%, with associated increases in type I (10.4%) and IIa (8.7%) fibres. The authors suggested that the smaller increase in type IIa fibres may be due to the fact that they adapt at a slower rate than type I fibres, possibly as a consequence of motor unit remodelling. Alternatively type I fibres are more sensitive to changes as they contribute more to force production in elderly muscle.(19)

There is a fast to slow shift in MHC isoform expression across all age groups following strength training, specifically a decrease in MHC IIx and a reciprocal increase in MHC IIa expression.(64;126;127) This indicates that type IIx fibres are readily transformed to a more metabolically active form (IIa) in response to heavy resistance training. Of particular note, MHC I expression did not change following 6 months resistance training in adults aged 60-75 years.(126) The reason for this is unclear.

When analysing pretranslational MHC mRNA, current evidence also supports a fast to slow shift, irrespective of age.(18;68;128) A 4 month endurance cycling program applied to subjects between 21-87 years of age increased the quantities of MHC type I and IIa mRNA by 63% and 99% respectively whereas type IIx mRNA decreased by 50%.(18) There were corresponding changes in MHC protein expression in younger subjects, unlike older subjects where protein content did not change. The authors suggested that gene translation into protein may be delayed or altered in older subjects.(18) Alternatively, a greater stimulus from

more intensive or longer programs may be necessary to produce changes in protein levels.(18) Balagopal et al also found an increase in levels of MHC I mRNA and a decrease in MHC IIx mRNA expression after 3 months resistance training.(68) However, there was a decrease in MHC IIa mRNA. Thus there appears to be agreement in relation to changes in expression of MHC I (increase) and MHC IIx (decrease) mRNA levels Further study is required to explain the differences in expression observed with MHC IIa transcription levels following training. O'Neill et al studied a shorter 7 consecutive day endurance training program using young male subjects.(128) While there was no change seen after the first session, a downregulation of MHC IIx mRNA was found before and after the final session indicating that changes in MHC IIx isoform expression occur early in response to training.

1.5.5 Adaptations in Muscle Atrophy and Hypertrophy Pathways

Five weeks resistance training increased IGF mRNA expression in the quadriceps muscle of elderly men, although there was no further upregulation when reanalysed at 12 weeks.(129) This supports the large increase in muscle IGF-1 protein levels (500%) and associated increase in quadriceps strength (260%) and muscle fibre CSA (10-13%) reported by Singh et al following 10 weeks resistance training in adults aged 72-98 years.(130) In vitro studies have shown that IGF-1 activates the PI3K/Akt/mTOR pathway inducing myotubule hypertrophy of cultured human muscle cells as well as an increase in MHC content.(131;132) Eight weeks of resistance training in young subjects increased muscle mass and was associated with increased phosphorylation of Akt and mTOR and decreased FOXO expression(93) Increases in IGF-1 levels following training are greater in younger subjects, giving an explanation for their greater fibre hypertrophy.(133) The hypertrophy pathway may also be more readily activated in subjects who participate in regular exercise as is seen by greater activation of PI3K in response to insulin.(134)

Recently Mascher et al demonstrated downregulation of MAFbx by 30% in muscle obtained 48 hours after a single bout of resistance training.(135) In contrast, MuRF-1

increased but was then downregulated by 30% following a second exercise session. Prolonged resistance training in young subjects increased expression of both E3 ligases with their subsequent down-regulation following detraining.(93) It is unclear as to why levels of MURF-1 and MAFbx do not decrease *following* prolonged training given they are associated with muscle atrophy. It may be that their levels are elevated to facilitate the normal turnover of an increased muscle mass.

Myostatin mRNA decreases within 24 hours following resistance training and remains downregulated with prolonged programs.(136-138) The effect is less in the sarcopenic muscle of older female subjects, potentially limiting hypertrophy in response to training.(136) However, changes in myostatin mRNA expression do not correlate with muscle strength or mass confirming that additional complex interactions are involved.(137;138) Kosek et al found increased MyoD mRNA concentrations in young and old subjects in response to 16 weeks resistance training, although fibre hypertrophy was more pronounced in young men.(121) It has been suggested this is due to greater activation and incorporation of muscle stem (satellite) cells into hypertrophying fibres in young men.(133)

1.6 FUNCTIONAL IMPROVEMENTS FOLLOWING STRENGTH TRAINING

Several *studies* on relatively healthy adults have found significant improvements in functional capacity following training. Power and gait velocity improved in older subjects after 4 months of PRT with levels comparable to untrained young subjects.(112) Much of the increases occurred in the first 8 weeks and was independent of muscle mass.(112) High intensity training for 6 months also reduced the time taken to rise from a chair in a cohort aged over 65 years.(139) Such improvements have been accompanied with decreases in surface EMG recordings indicating a reduction in physical demand.(112)

A 12 month group-based exercise program resulted in improved walk and chair rise times.(140) The gains were not maintained over the following 6 months when subjects were required to train unsupervised at home. Fiatarone et al reported a 48% increase in gait speed

following an 8 week high intensity resistance training program in very elderly subjects and reduced dependence on walking aids.(108) A later study by the same group used a PRT program on a larger aged cohort finding improvements in gait speed (11.8%) and stair climbing power (28.4%).(141) A task-specific rehabilitation program which focused on rising from a bed or chair produced gains of 11-20% in adults over 65 years with additional beneficial effects on strength and balance.(142)

Not all studies have found gait speed to significantly improve despite gains in muscle strength.(139) It may be a less sensitive marker of functional capacity which is affected by the assessment protocol chosen. Gait velocity can be assessed either by timing a subject over a set distance or alternatively recording the distance walked over a set time. Both parameters vary between studies. For example, a study on subjects aged over 75 years who received 3 months resistance training found no improvement on gait velocity or chair rise times despite increasing muscle strength by 13-21%.(143) The researchers suggested their cohort were all very healthy, and therefore not representative of this elderly population.

Chapter II

Is there a role for neuromuscular electrical stimulation in knee osteoarthritis?

2.1 KNEE OSTEOARTHRITIS

Osteoarthritis is considered the main cause of chronic disability in adults aged over 65 years.(144;145) Between 1995 and 2008 its prevalence increased by 29%, and now affects 27 million people in the USA.(146) The Framingham osteoarthritis study found the radiological incidence of knee OA to be 1.8% in women and 1.0% in men with half becoming clinically symptomatic.(147) Bilateral disease is more common than unilateral OA (5% vs. 2%), and has a greater association with obesity than trauma.(148) Of all adults with knee OA aged over 65 years, 55% have bilateral involvement.(149)

2.2 QUADRICEPS DYSFUNCTION AND KNEE OSTEOARTHRITIS

There is no significant difference in strength between their dominant and non-dominant legs of young and elderly healthy individuals.(11;26;96;102) In contrast, asymmetrical weakness is a common feature of knee OA with disuse atrophy and muscle activation failure producing unilateral strength deficits of up to 50%.(149-155) The exact relation between knee OA and strength decline has not been clearly defined. It has been proposed that quadriceps weakness is an aetiological factor in the development and progression of knee OA since small gains in strength reduce its incidence by 20-30%.(149;156) There is a recognised association between knee OA and meniscectomy. Only quadriceps strength declines after arthroscopic meniscectomy, whereas hamstring and adductor muscle strength is preserved.(157) Conversely, Brandt et al found no relation between baseline quadriceps strength and symptomatic knee OA progression, and no significant change in quadriceps strength despite radiological disease progression.(158) The equivocal findings may be methodological since the severity of knee OA has been determined arthroscopically by some and radiologically by others. Since the association between plain knee radiology and

clinical symptoms is often weak, their value should be applied with caution.(159;160) Quadriceps strength is a better determinant of pain and functional disability associated with knee OA than radiographic change.(161) Accordingly, adults with symptomatic knee OA are weaker compared to asymptomatic subjects despite similar stages of disease when graded radiologically.(162)

2.2.1 Muscle Activation Failure and Disuse Atrophy

Muscle weakness in knee OA has been attributed to arthrogenous muscle inhibition (AMI) causing a failure of muscle activation. Altered afferent signals from the arthritic joint decrease efferent α-motoneuron output to the quadriceps muscle.(163-165) Adults with knee OA may also reduce their voluntary central neurological drive in an attempt to minimise knee pain.(166) Clinically this manifests as a reduction of both voluntary muscle activation and force generating ability.(152) Although elderly subjects have a baseline activation deficit, it is approximately 20% greater in those with knee OA.(152;164) Greater levels of muscle activation failure in knee OA is associated with reduced functional performance.(163;164)

Significant quadriceps muscle atrophy occurs in association with knee OA and is an important determinant of strength.(154;167;168) Reductions in lean muscle mass by up to 12% in the affected limb has been reported in both early and advanced stages of disease.(154;168;169) There is disagreement regarding the level of interstitial fat and connective content in the quadriceps muscle of subjects with OA. Rasch et al reported increased levels in subjects with hip OA, whereas Petterson et al found no difference in knee OA.(154;170) This discrepancy may reflect joint specific changes. Rasch et al assessed muscle mass using CT whereas Peterson et al used MRI. It is not possible to directly compare the two imaging modalities, although MRI is generally considered superior when evaluating soft tissue.

Petterson et al reported that muscle activation failure contributed more to quadriceps weakness than muscle atrophy in subjects with advanced knee OA, suggesting that activation deficits may arise first and potentiate atrophy.(154) Attempting to define this further, Pap et

al reported that although subjects with advanced disease have less activation failure than those with moderate disease (23% vs. 29%), their maximum force generating ability was lower indicating a reduction in overall strength capacity.(152) Conversely, Lewek et al found muscle atrophy to be the principle cause of quadriceps weakness in OA.(166) However, no information was given regarding disease severity.

2.3 BIOMECHANICAL FACTORS ASSOCIATED WITH KNEE OSTEOARTHRITIS

Obesity, joint laxity, instability and malalignment as well as altered proprioception have been associated with knee osteoarthritis.(145;164;171-173) Subjects with knee OA are more obese with its effect more pronounced in women.(149;174) Obese individuals with and without knee OA have up to 20% greater absolute quadriceps strength than their lean counterparts possibly due to their increased weight acting as a training stimulus.(167;175) However, this is reversed when adjusted for body mass, producing a relative deficit of 35%. In subjects with knee OA the deficit is even greater.(149) Given the relation between strength and BMI, it has been suggested that reduced strength relative to body weight rather than absolute strength may be considered a risk factor for OA, particularly in women.(167)

Sharma et al found that BMI relates to radiological OA severity in varus rather than valgus knees.(173) The authors were unable to comment on the aetiological potential of malalignment due to the cross-sectional study design. It appears that varus knees increase the potential for disease progression in overweight subjects. The same group also described greater coronal laxity in both knees of adults with knee OA compared with age-matched controls.(176) While this is certainly expected in involved limbs due to articular cartilage loss decreasing tibiofemoral stability, the finding of increased laxity in the contralateral limb raises the possibility of joint laxity precipitating incident OA and disease progression.

2.4 FUNCTIONAL DISABILITY IN KNEE OSTEOARTHRITIS

Adults with knee osteoarthritis have significant functional limitations when assessed using gait, chair-rise and stair-climbing tests.(162;164;177) Subjective impairment has also been described using validated instruments such as the Stanford Health Assessment Questionnaire and the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC).(149;159;178)

Eccentric (antigravity) quadriceps action helps to control the rate of knee flexion at the end of the swing phase during normal gait.(156) Eccentric force is reduced more than concentric or isometric strength in subjects with knee OA.(179) Compensatory measures include reducing knee angular velocity and range of motion during gait.(180;181) Individuals with knee OA increase the rate of loading in the contralateral limb after heel-strike and apply lower vertical force during push-off compared to healthy adults.(180) Gait velocity cadence and stride length are reduced whereas stride width and knee adduction are increased.(181;182)

In addition to asymmetric strength loss, there is a reduction of quadriceps muscle contractile velocity (15-128%) and decreased endurance (up to 200%) in subjects with knee OA compared to healthy age-matched controls.(155;183) Obese subjects are also less fatigue resistant, reducing further the capacity of subjects with knee OA to perform activities requiring consecutive motor actions such as stair-climbing and uphill walking.(175)

Quadriceps weakness adversely affects lower limb balance.(184) Hortobagyi et al studied the mechanisms underpinning force control in knee OA and found that impaired knee joint proprioception was more pronounced with knee extension.(179;185) In addition, osteoarthritis subjects had greater difficulty in controlling force production at submaximal strength levels. Greater hamstring coactivation is required to assist knee balance and stability.(149;181) Surface EMG analysis recently confirmed this effect in subjects with

moderate knee OA during walking and stair-climbing tasks.(185) OA progression may be potentiated through increased joint contact forces producing articular cartilage damage.(181) By maintaining knee kinematics similar to those of younger adults, it is possible for older subjects to preserve normal knee *function* despite quadriceps weakness potentially decreasing the rate of joint degeneration.(181)

2.5 EXERCISE TRAINING IN KNEE OSTEOARTHRITIS

Exercise therapy has been shown to be effective in improving quadriceps strength and function in subjects with knee OA without perpetuating disease progression.(161;178) In addition, subjects with varying disease severity have reported reductions in knee pain between 13-40% following exercise training.(150;186)

2.5.1 Functional Capacity

Fisher et al performed several studies assessing the efficacy of 2-4 months exercise training on subjects with knee OA.(150;186-189) Gains in quadriceps strength ranged from 8% to 55% with the greatest improvements often occurring in the initial 4 weeks. Corresponding increases in quadriceps muscle activation ranging from 5-20% have also been found and are associated with improvement in joint proprioception and disability.(190) Quadriceps and hamstring muscle endurance also improves *in response* to exercise training by 22-42% and 18-43% respectively.(150;186;189;191) Reported improvements in angular velocity range from 34-40%.(186;187) Given that subjects with knee OA report increased muscle fatigue, especially in the presence of fixed flexion deformities, it is clear how such gains can positively affect functional capacity. Fisher et al found a 21% improvement in walking speed with a corresponding QFM strength gain of 55% in response to exercise training, although knee kinematics did not change.(186) It has been suggested that a lower aerobic capacity is a secondary effect of muscle dysfunction in subjects with knee OA and improves following a program focused on muscular rehabilitation.(189)

While the majority of recent evidence supports exercise training as a therapeutic modality in knee OA, the Framingham study reported that physical activity increased the risk of OA.(171) However this was only associated with adults who were considered very active.

2.5.2 Compliance

There is a dose-response relation between exercise compliance and improvements in subjective pain and function.(192;193) Adherence with exercise programs is problematic in subjects with knee OA. A multicentre study from north America compared the effects of 18 months aerobic and resistance training programs in men and women greater than 60 years of age.(194) Both training modalities produced comparable functional gains with similar compliance rates (68-70%). Studies of shorter durations have found compliance rates ranging from 47-78%.(187;195) Patients may consider regular formal exercise a burden, especially when their symptoms are reduced.

2.5.3 Financial Implications

Lack of time and financial constraints including necessary healthcare facilities and manpower may influence the adoption of exercise training for the treatment of knee OA. A recent study evaluated the economic implications of a rehabilitation program consisting of exercise, self-management and coping strategies in adults with chronic knee pain compared to standard primary care.(196) Costs were £314 per person for individual treatment programs compared to £125 per person involved in group rehabilitation. The therapeutic effectiveness of both programs was superior to standard care.

Home-based rehabilitation programs may be an effective strategy to promote long-term adherence An early study from Fisher et al failed to find significant functional improvements despite ia 35% increase in isometric quadriceps strength following a 3 month home-based training program.(187) The drop-out rate was 50%. More recent studies using

larger sample sizes have found significant functional improvement in patients with knee OA following 8 to 10 weeks of home-based training.(197;198)

2.6 NEUROMUSCULAR ELECTRICAL STIMULATION

The use of electrical current to elicit muscle contractions has intrigued scientists and physicians alike for several centuries where its use in medicine was initially coined electrotherapy. One of the notable contemporary uses is in cardiac pacemakers. The clinical application of neuromuscular electrical stimulation (NMES) describes its use to evoke contractions of normally innervated but weakened muscles through stimulation of intact peripheral nerves. Functional electrical stimulation (FES) represents its chronic use, typically in the management of para/quadriplegic patients following spinal cord injury where it facilitates, amongst others, gait rehabilitation.(199) Current therapeutic applications of NMES usually involve direct transcutaneous application over a selected muscle rather than indirect stimulation of a motor nerve, often to limit atrophy in the early stages of rehabilitation. NMES also gained favour with athletes where it was hoped it would confer competitive advantage.(200)

2.6.1 Recruitment Patterns

Voluntary muscle contraction is associated with a graded pattern of motor unit recruitment. This has been summarised by the Henneman "size principle" whereby motor units and their corresponding motoneurons are progressively recruited in the inverse order of their neuron size (Figure 2.1).(201)

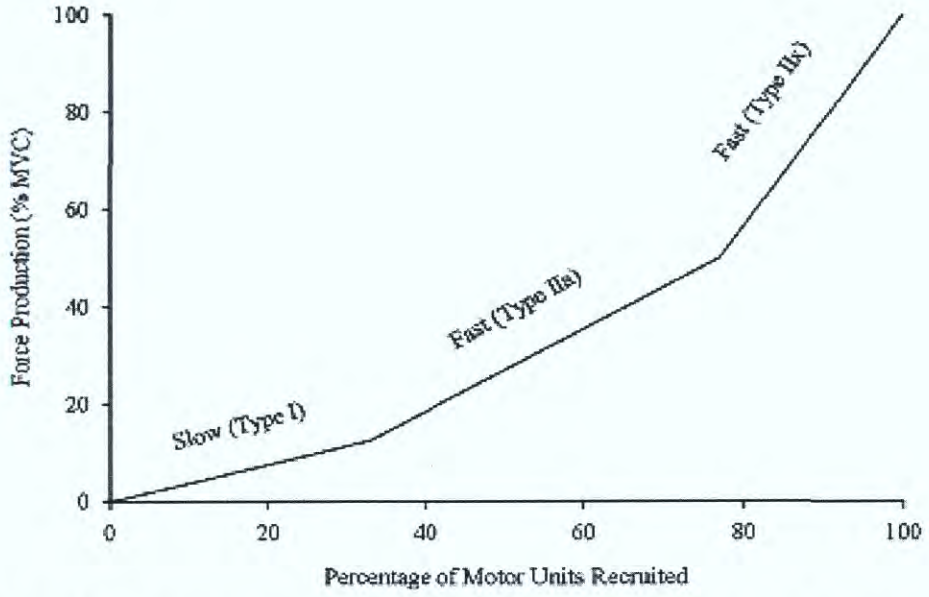


Figure 2.1. Orderly recruitment of motor units during voluntary activation described by Henneman et al.(201) MVC: Maximal Voluntary Contraction Force

The ordered recruitment pattern is explained in part by Ohm's Law (V=IR) and membrane impedance (resistance). Impedance is proportional to the resulting membrane potential and in turn the excitatory post-synaptic potential (EPSP). Since smaller neurons have greater membrane impedance they develop larger changes in membrane potential in response to a given pre-synaptic current (excitatory drive) producing greater EPSP's. Since an action potential (AP) is produced when the summation of these EPSP's achieves the necessary threshold level, small motoneurons can fire an AP in response to lower levels of presynaptic input. Accordingly type I (slow twitch, less fatigable) fibres are activated at low levels of input stimulus followed by larger, more powerful, type II (fast twitch, more fatigable) fibres (IIa then IIb) as neural drive increases.

An electrical stimulus, when applied directly to motor nerves in vitro, will choose the path of least resistance. Since large diameter axons have lower membrane impedance they tend to be preferentially activated.(202) Early evidence supported a reversed order of fibre

recruitment in response to NMES.(203-205) Gregory and Bickel recently examined published physiological, metabolic and mechanical data, arguing that transcutaneous NMES actually recruits motor units in a "non-selective, spatially fixed, and temporally synchronous pattern".(206) Feiereisen et al compared the recruitment patterns of voluntary contraction and NMES on the recruitment threshold of approximately 300 MU's taken from the tibialis anterior muscle finding a reversal of recruitment in only 30%.(207) Knaflitz et al found similarly increased conduction velocities and frequency with in response to voluntary and NMES induced contractions.(208) Several studies found increased muscle fatigability with NMES which may have indicated a reversed order of recruitment as type II fibres are more fatigable. Gregory and Bickel offered an alternative explanation, suggesting that during voluntary contractions alternative recruitment patterns allow recruitment of different MU's when initially recruited fibres fatigue.(206)

Sinacore et al investigated the effects of NMES applied to the quadriceps muscle of a single subject on myofibre glycogen utilisation.(204) There was greater depletion of type II fibre stores than type I leading the authors to suggest preferential activation of type II fibres. Since type II fibres have higher energy demands and increase glycogen phosphorylase activity it is possible that differences in fibre metabolic activity rather than recruitment patterns could explain the results.(206) Furthermore, if there was a strict reversal of recruitment, glycogen utilisation in type IIb fibres would have been greater than type IIa fibres, although this was not the case.(204) Kim et al performed a similar study using 7 subjects.(209) NMES resulted in greater glycogen depletion than voluntary contractions but all fibres were similarly affected refuting a reversed order of recruitment.

A study of twitch contractile velocity, force-frequency and fatigue of the quadriceps muscle found no significant differences using stimulation intensities of 20%, 50% and 80% MVIC indicating a less orderly recruitment pattern.(210) Using the twitch interpolation technique to compare contractile velocity of voluntary and NMES induced contractions Jubeau et al found that MU's were recruited in a random order during NMES.(211) Factors that influence the physiological effects of NMES in vivo include skin impedance and subcutaneous fat (distance from the stimulating electrode) as well as axonal orientation within muscle.(202;206;208) Despite a random order of MU recruitment, it does appear that NMES can recruit high threshold (fast twitch fibres) more readily than voluntary activation.(202)

2.7 VARIABLES AFFECTING TORQUE PRODUCTION

2.7.1 Stimulation Parameters

It is still unclear as to what are the optimum stimulation characteristics for NMES training. Variables that can be manipulated include pulse frequency (rate), duration and amplitude (intensity) as well as current waveform. Ultimately there needs to be a compromise between torque generation and patient comfort. The difficulty in interpreting data is further compounded since many studies have been performed on young healthy subjects in conditions that do not replicate true clinical scenarios. A recent review by Vanderthommen and Duchateau reported that the most commonly employed stimulation parameters are: biphasic, rectangular, symmetrical waveform with a pulse duration of 0.1-0.5 milliseconds and a low frequency of 50-100Hz.(202)

Pulse durations that are very short (~0.1 msec) require higher stimulation intensities to achieve muscle activation. While this may be acceptable for small muscles such as wrist extensors, larger muscle groups such as the quadriceps require durations of approximately 0.3 msec. Pulse frequency determines the rate at which MU's fire action potentials, and like stimulation intensity, is relative to the amount of force generated.(212) A stimulation frequency that is too high will result in more rapid muscle fatigue.(213;214) Fatigue has been defined as a decrease in the force-generating ability of a muscle resulting from recent activity and can be related to utilisation of the muscle fibres metabolic and energetic reserves.(214) It may also relate to insufficient neurotransmitter release at the synaptic junction occurring at high stimulation frequencies. Therefore low frequency, high intensity stimulation is preferable for NMES training.(214) Clinical stimulators, both mains and battery powered, do not apply continuous current. "Off-times" help prevent muscle fatigue during strength training permitting effective muscle contractions over extended periods.(214)

2.7.2 Electrode Size and Orientation

Consideration must be given to electrode position and size. If the electrodes are placed to close together, current may only pass through the soft tissues. Placing the electrodes further apart permits activation of the deeper tissues, specifically underlying muscle. At a given stimulation intensity, the current passing through the tissues increases as the electrode size decreases, reducing patient tolerance due to higher current density adversely stimulating cutaneous sensory receptors. The use of larger electrodes is preferable since they result in less discomfort and confer greater tolerance.(215) In turn, larger stimulation intensities can be tolerated resulting in increased torque generation. Conversely,

Lieber and Kelly reported that electrode size did not affect force generation.(216) A major limitation of their study was that the three electrodes were composed of different materials. Although larger electrodes can target more muscle motor points, they utilise greater amounts of electrical energy to cause a contraction. .(215) Smaller electrodes, if positioned appropriately can be equally effective.

2.7.3 Subject Tolerance

There is considerable variability between subjects in the amount of torque produced at a given stimulation intensity as well as differences in tolerance for higher stimulation intensities.(202;216) The reasons for this are unclear. Although pulse frequency and stimulation intensity can be lowered to comfortable levels, they may be inadequate in producing effective torque generation. Elite athletes can generally tolerate higher levels of intensity given that they are accustomed to discomfort associated with high training levels. Obese subjects require greater levels of stimulation since the current has to pass through the adipose layer before it can reach the muscle and elicit a contraction.(217) Male subjects experience less pain and can tolerate higher levels of stimulation intensity than females.(218;219) Following repeated sessions of NMES, both genders can be conditioned to tolerate higher levels of stimulation intensity.(218)

2.8 NEUROMUSCULAR ADAPTATION IN RESPONSE TO NMES

Volitional resistance training induces substantial changes in neural control, motor unit characteristics and myofibre morphology (Chapter 1). Given that NMES causes muscle activation by direct stimulation of motor units in a more random order it would be expected that different neuromuscular adaptations would occur. Furthermore, since central neural drive is not directly involved one could postulate that NMES would not result in significant changes in the neural system. It is only relatively recently that researchers have attempted to elucidate the mechanisms of neuromuscular adaptation in response to NMES.

2.8.1 Neural Adaptation

There is increasing evidence that NMES can induce significant neural adaptations. Improvements in muscle activation have been found following 4-6 weeks NMES applied to the plantar-flexors and quadriceps musculature.(220-222) Gondin et al used the interpolated torque technique (ITT) to assess voluntary muscle activation after an 8 week NMES program applied to the quadriceps femoris of young healthy men.(223) Isometric strength increased by 27% in association with improved quadriceps activation by 6% from a baseline of approximately 90%. Most of the improvement in muscle activation occurred in the first 4 weeks and greater gains in strength were associated with greater activation deficits at baseline. Surface EMG analysis of the individual extensor muscles demonstrated increased activity in the mono-articular vastus lateralis and vastus medialis muscles but not the bi-articular rectus femoris muscle, possibly reflecting its lack of direct stimulation during NMES training.(223) Only the right limb was assessed and flexion strength, biceps femoris surface

EMG and hamstring co-activation did not change.(223) Therefore neurological adaptations appeared to be confined to the quadriceps muscle.

However, Maffiuletti et al reported increased hamstring coactivation (from 6.8% to 9.5%) in a single young sedentary subject following 4 weeks of unilateral NMES training.(220) Isometric quadriceps strength increased by 12% in the trained, non-dominant limb,and 8% in the contralateral limb indicating cross-education. This phenomenon has been previously reported with both NMES and volitional exercise, and although the exact mechanisms for this are unknown, adaptations at the level of the spinal cord have been implicated.(205;224-227) Baldwin et al demonstrated spinal motor neuron recruitment by demonstrating that torque could be evoked at intensities below the motor threshold.(228) There is also evidence of increased cortical activity in response to NMES determined with functional MRI which is dependent on stimulation intensity.(229) Han et al showed that activity in the contralateral primary sensory cortex was increased to a greater extent than the motor cortex as well as evidence of bilateral motor activation.(230) Clearly, NMES should not be considered as a modality that affects only peripheral neurons. It may indirectly, possibly due to efferent cutaneous feedback, involve additional pathways.

2.8.2 Muscular Adaptation

In addition to changes in neurological control, Gondin et al found that 8 weeks NMES significantly increased quadriceps CSA by 6%, with most of the improvement occurring in the final 4 weeks.(223) There was selective hypertrophy since CSA of the three vastii muscles increased whereas rectus femoris CSA did not change significantly. This is in keeping with their

findings from surface EMG analysis which found that rectus femoris activity did not change, indicating preferential activation of the quadriceps muscles with NMES.(223) It also appears that the time course of neuromuscular changes is similar to that found with volitional exercise. Increases in muscle strength in the first 4 weeks of NMES training are due to neural adaptation while muscular changes account for further increases in strength with longer training durations.(221;223;231)

To date, only one study has assessed the effects of NMES on individual muscle fibre CSA and specific force as well as MHC protein composition.(220) Four weeks of isometric quadriceps NMES training increased type I fibre CSA by 27% whereas type IIa fibre CSA increased by only 6%. Type IIb fibre CSA was not assessed. Expression of MHC IIx decreased by 28%, MHC IIa levels increased by 22% and MHC I did not change, indicating a fast to slow shift as has been observed with resistance exercise (Chapter 1). Most studies have shown that NMES does not effect the contractile properties of muscle.(221;223;231;232) Only one study found contractile velocity to be reduced, although the effect was only transient as it returned to baseline levels after 4 weeks of detraining.(220)

2.8.3 Detraining

The neuromuscular changes that occur in the detraining period following NMES training have not been extensively studied. Studies that have used NMES to strengthen the gastrocnemius have generally reported preservation of neural drive and maintenance of muscle strength for up to 6 weeks after completion of training.(222;227) Studies on the detraining effects on quadriceps strength are variable. One study reported continual

improvement in muscle strength by 30% four weeks after cessation of quadriceps NMES training.(220) This was essentially a case report as only one subject was studied. Mauqueste et al found that the increases in quadriceps strength after 6 weeks of NMES training were maintained after 6 weeks detraining.(233) In contrast, Gondin et al found quadriceps strength decreased after detraining.(231) This was associated with both a reduction in muscle activation and muscle atrophy although the neural changes occurred first.

2.9 QUADRICEPS STRENGTH TRAINING USING NMES

There have been many reports where NMES has been used to increase quadriceps strength of young healthy subjects, athletes, sedentary subjects, and elderly cohorts. It is difficult to compare studies effectively due to differences in stimulation parameters, fitness of subjects, number and duration of training sessions, as well as variations in outcome variables. Most interventions last 4-5 weeks involving 20-25 sessions, each lasting 10-30 minutes.(202) Current evidence suggests that compared with doing no exercise, NMES can effectively increase strength in subjects with and without quadriceps impairment although strength gains depend on the intensity of the electrically induced contractions during training.(200;202;224;234;235)

NMES may offer no advantage when compared with volitional exercise programs.(200;234) Although Hortobagyi et al found that 6 weeks NMES increased strength more than an eccentric training program, others have found NMES to be similar or less effective than isometric training in young healthy subjects.(224;232;236) Similar studies on elderly male and female subjects found similar strength gains after 4 to 6 week NMES or

voluntary exercise programs.(237;238) Caggiano et al found that NMES produced greater strength gains in sedentary elderly men than those who participated in regular exercise.(238) A 4 week postoperative NMES has not been shown to enhance recovery any more than standard exercise rehabilitation following anterior cruciate ligament (ACL) reconstructive surgery.(239)

Since NMES and volitional exercise induce contractions by different mechanisms they are often used as complementary therapies. This involves either the superimposition of electrical stimulation onto voluntary muscle contractions (superimposed technique) or alternatively combining the two training modalities separately (combined technique). Superimposition of NMES is no better than voluntary exercise alone in the training of young or elderly healthy subjects, although it may be superior than exercise rehabilitation in the recovery of muscle strength and volume after surgery.(240) In athletes, the combined technique induces greater improvements in strength and the performance of complex tasks compared to voluntary exercise alone.(241) There is evidence that the combination of NMES and voluntary training may adversely affect balance, certainly in elderly females.(242) The mechanism for this is unclear It has been suggested that the combination technique is more effective compared with standard exercise protocols when used as a rehabilitation modality after knee ligament surgery.(243) However several studies have found no difference.(244;245)

2.10 THE USE OF NMES IN KNEE OSTEOARTHRITIS AND TOTAL KNEE ARTHROPLASTY

The relative preservation of muscle strength and CSA following NMES training has led to the suggestion that NMES may have a role before, during and after periods of

immobilisation to minimise declines in muscle strength and mass.(220;231;234) When electrodes were applied to the skin through holes in casts, NMES reduced the detrimental effects of immobilisation on both quadriceps and plantarflexor strength and muscle mass.(246) Bax et al also recommended using NMES in situations where compliance with volitional training is low.(234) As subjects with knee osteoarthritis have marked quadriceps weakness due to muscle activation deficits and disuse atrophy it would be expected that NMES would have a beneficial effect and possibly result in better compliance. A study of elderly patients with knee OA utilised a portable NMES program over 12 weeks (36 sessions) and found a 9% increase in quadriceps strength with associated improvement in functional capacity (247) Adherence for the NMES group was slightly better than the education control group (85% versus 78%). When compared with voluntary exercise in a similar osteoarthritic cohort, NMES was deemed to be equally as effective in reducing pain, increasing quadriceps strength, and improving both subjective and objective performance measures.(248) Similar effects have been reported in subjects with rheumatoid arthritis.(249) It is not known, however, as to what effect NMES has on muscle mass in patients with knee osteoarthritis.

Volitional strength training alone may not be sufficient to fully restore quadriceps strength after total knee arthroplasty (TKA) as deficits between involved and uninvolved knees can persist for up to 2 years.(52) For example, the affected quadriceps muscle of a 62 years old male subject was 26% weaker compared to his unoperated limb 12 months after TKA.(250) Following 6 weeks intensive combination therapy, quadriceps strength increased by 25% while muscle activation improved from 83% to 97%. The combination of NMES with volitional exercise immediately after TKA can reduce the length of hospital stay and improved extensor

lag and walking speed to a greater extent compared with standard rehabilitation.(251;252) Mintken et al described greater quadriceps activation and strength at 3 weeks post-TKA compared to preoperative levels in a single patient using 3 weeks combination rehabilitation. (253) There were continued improvements in subjective (SF-36) and objective (stair-climb and chair-rise tests) functional measures when reassessed at 12 weeks postoperatively. Given the improvements in strength, mass and function seen in patients with quadriceps impairment it is reasonable to suggest that preoperative NMES may reduce their decline in the early postoperative period.

Chapter III

General Methodology

3.1 EXPERIMENTAL DESIGN OVERVIEW

Study 1: Quadriceps femoris neuromuscular electrical stimulation in knee osteoarthritis: effects on muscle strength and clinical function

Sixteen healthy adults with advanced knee osteoarthritis were assigned to either intervention (NMES) or control (Con) groups for an 8 week training program. The first two weeks constituted a conditioning period. Strength was assessed at baseline, week 2, week 5 and week 8. Functional capacity (timed walk, chair-rise, and stair-climb), and subjective outcome measures (WOMAC, SF-36, and Oxford Knee Score) were evaluated at baseline and week 8.

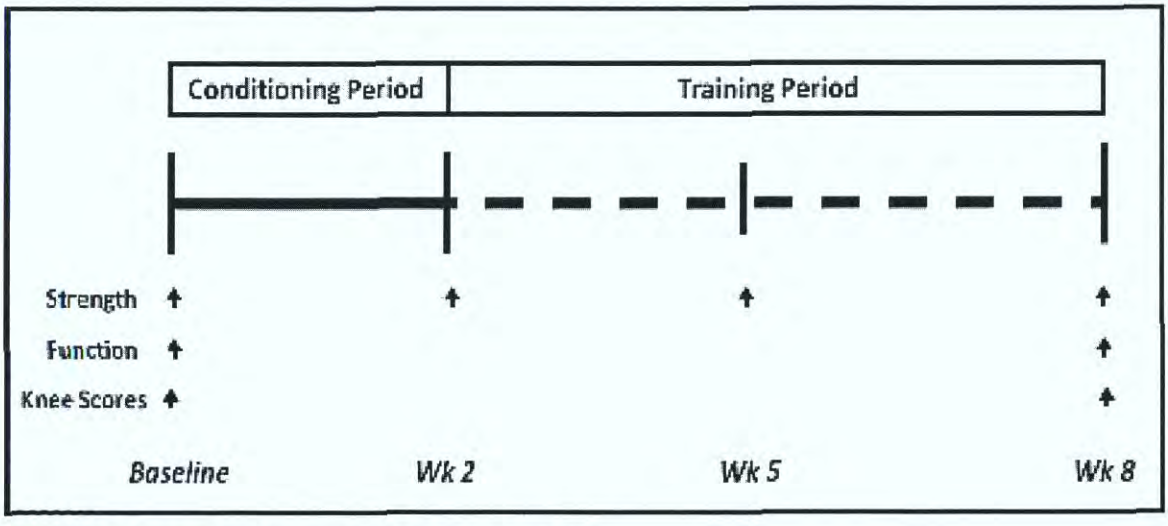


Figure 3.1. Schematic of experimental design for study 1

Study 2: Quadriceps femoris neuromuscular electrical stimulation in knee osteoarthritis: the mechanisms associated with strength gain

Fifteen healthy adults with advanced knee osteoarthritis were assigned to either intervention (NMES) or control (Con) groups for an 8 week program. Muscle strength and CSA were assessed at baseline and week 8. From this data muscle specific force (F^0) was

calculated. Skeletal muscle biopsies were obtained from the *m.vastus lateralis* at baseline and week 8 for analysis of gene expression

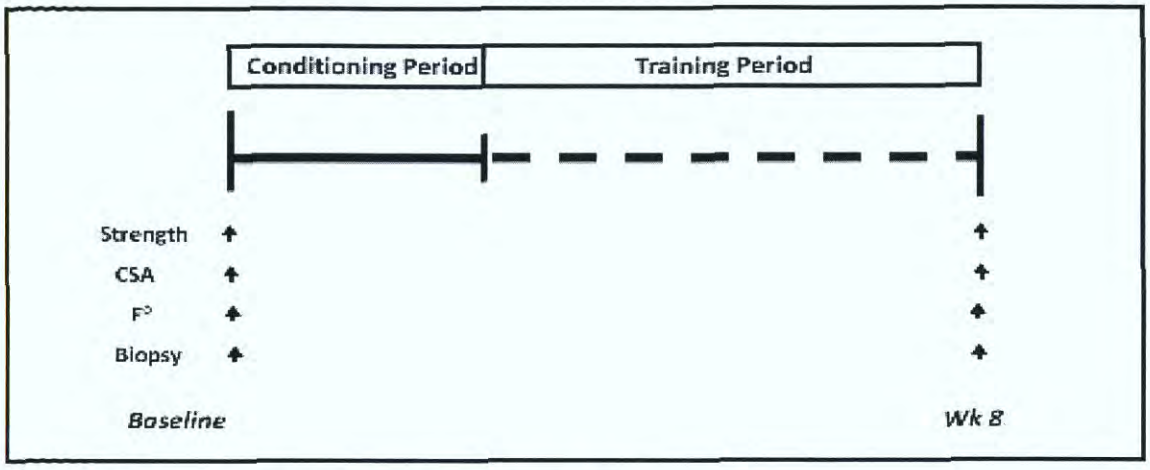


Figure 3.2. Schematic of experimental design for study 2

Study 3: Effects of a prehabilitation program in total knee arthroplasty using neuromuscular electrical stimulation

Fourteen healthy adults undergoing total knee arthroplasty were assigned to intervention (NMES) or control (Con) groups for an 8 week preoperative training program. Strength, functional capacity and subjective well-being were assessed at baseline and preoperatively (week 8), with two further assessments at weeks 6 and 12 postoperatively. Muscle CSA was determined at baseline, preoperatively, and at 12 weeks post-TKA.

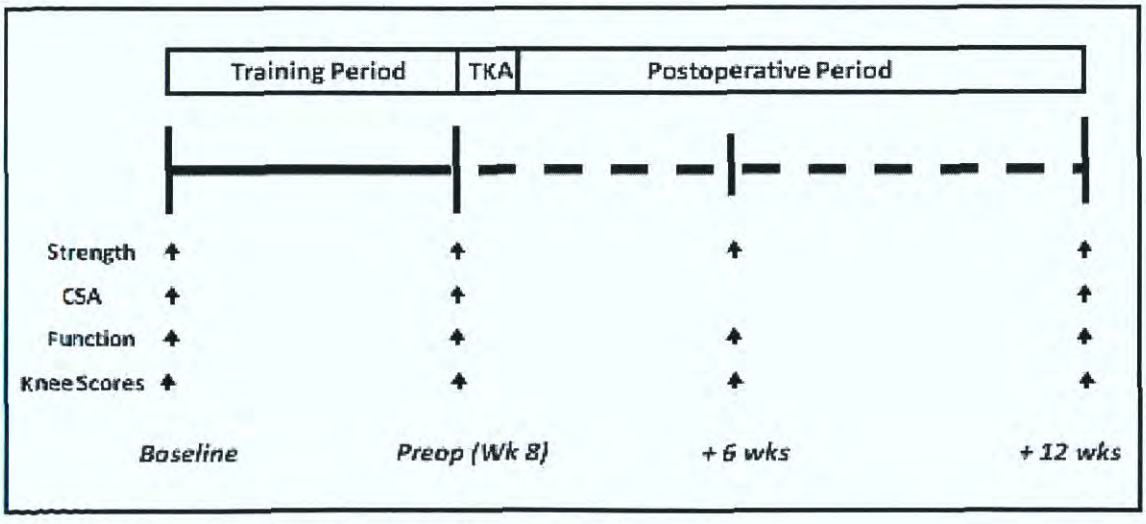


Figure 3.3. Schematic of experimental design for study 3

3.2 POWER ANALYSIS

An interim power analysis was performed after the first 5 subjects were enrolled using baseline data from the timed chair rise test (mean, 10.0; SD, 1.27). From a proposed difference between groups of 25% (standardised difference, 1.97) and a power of 0.85 (alpha = 0.05, two sided) a normogram was used to determine the sample size (n).(254) Based on this information, we calculated the total sample size necessary to determine a difference in objective function to be 12.

3.3 PARTICIPANT RECRUITMENT

Patients with severe knee osteoarthritis scheduled for Total Knee Arthroplasty in Cappagh National Orthopaedic Hospital were recruited. The study was approved by the Cappagh National Orthopaedic Hospital Ethics Committee and conformed to the Declaration of Helsinki (Appendix I). Subjects who fulfilled the selection criteria (Table 3.1) determined by reviewing the records from the pre-admission assessment clinic (PAC) were approached. A plain language information sheet detailing the purpose, procedures, risks and duration was provided to individuals expressing an interest in the study (Appendix II). Informed consent was obtained from each subject willing to participate.

3.4 RANDOMISATION

Participants were randomly assigned to one of two groups: intervention (NMES) or control. Block randomisation was used in a 3:2 ratio using random numbers. The resulting randomization list was put into sealed opaque envelopes with their sequential order written on them. A member of the nursing staff of Cappagh Orthopaedic Hospital not involved in the trial recruitment process kept the envelopes in a secure location. Allocation to a group was only provided when a suitable, eligible subject consented to participate.

Table 3.1. Selection criteria

Inclusion Criteria
• TKA for unilateral tricompartmental knee OA
• Ambulatory Patients
• Scheduled for Total Knee Arthroplasty for Primary knee OA
Exclusion Criteria
• Unicondylar TKA
• Morbid Obesity (BMI > 40)
• Uncontrolled Hypertension
• Anticoagulant therapy
• Neurological disorder
• Malignancy
• Inflammatory arthritis
• Implanted pacemaker or defibrillator
• Dermatological conditions affecting the thigh
• Recent participation in an exercise or strength training program
• Inability to walk unassisted
• Severe cognitive impairment
• Other lower limb impairment affecting function including amputation

3.5 INTERVENTION – NEUROMUSCULAR ELECTRICAL STIMULATION (NMES)

Subjects assigned to the intervention group received a total of 8 weeks home-based neuromuscular electrical stimulation training applied to the quadriceps femoris muscle (QFM) of the affected knee. The duration of each session was 20 minutes.

3.5.1 Muscle Stimulator

A portable (battery powered), garment based neuromuscular electrical stimulator (KneeHAB II, Bio-Medical Research, Galway, Ireland) provided the external stimulus to elicit QFM contraction (figure 3.4). There are 2 channels on this device (medial and lateral) with a delay of 1 second between their activation at the start and end of the contraction sequence to

ensure patellofemoral stability. The control unit, attached directly to the garment, activated the stimulator and modulated stimulation intensity.

3.5.1.1 Stimulation Parameters

The stimulation characteristics of the KneeHab II unit are:

- Symmetrical, bi-phasic, square waveform
- Pulse width/duration of 100 and 400μs (for channels 1 and 2 respectively)
- Frequency of 50 pulses per second
- Maximal intensity of 18mA

Figure 3.4. Kneehab stimulator

Contraction time (ON) was 5 sec with 10 sec relaxation (OFF) [1:2] excluding 1 sec ramp-up and 0.5 sec ramp-down. Thus the total cycle length was 16.5 sec. This provided a total ON time of 6.06 min in each 20 min session.

The stimulus was provided by a "Multipath" system. This opens pathways between the electrodes for preset periods within each pulse, directing current flow to either medial or lateral electrodes, hence permitting multiple pathways for current flow. It is proposed by the manufacturer that this will allow greater stimulation intensities to be tolerated by a subject and produce greater contraction force while minimising muscle fatigue.

3.5.1.2 Surface Electrodes

Four reusable self adhering hydrogel electrodes (Axelgaard, Fallbrook, CA) were secured to the inside of the garment with placement over the vastus medialis and vastus lateralis proximally and distally.

The surface areas of the four electrodes are:

A: 194 cm^2 C: 83 cm^2

B: 74 cm^2 D: 66 cm^2

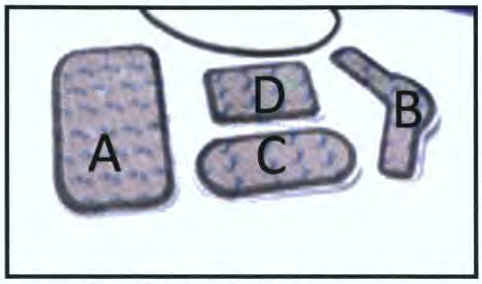


Figure 3.5. Surface electrodes

The electrodes consist of a silver printed carbon layer dispersive media (conductive vinyl layer) sandwiched between a skin-specific and belt specific hydrogel layers. The device only provided stimulation when all electrodes were in contact with the subjects' skin permitting an intact circuit. It would not function if the electrodes become loose or detached from the garment.

3.5.2 NMES Training Program

Subjects assigned to the NMES group received instruction on application of the device to the thigh of the affected limb and usage of the stimulator according to manufacturer guidelines. In addition, they were supplied with clear written instruction on the device controls and the NMES training program schedule (Appendix III). All NMES sessions were performed in the sitting position with the knee flexed to 60 degrees, the foot flat on the floor, and toes against a wall to permit isometric muscle contraction. Cardboard strips, cut with an angle of 60 degrees, were provided to assist with this. All training sessions were performed unsupervised as a home-based program.

3.5.2.1 Conditioning Period

Neuromuscular electrical stimulation may cause moderate discomfort at higher stimulation intensities. Alon et al have suggested a conditioning period of up to 2 weeks necessary for subjects to develop sufficient tolerance, thereby ensuring sufficient effective torque generation during the subsequent training period.(218) The initial 2 week period involved training with NMES on alternate days providing 7 sessions in total. The principal aim

was for subjects to gradually increase stimulation intensity to their maximum tolerated level aiming to achieve a level considered necessary for effective training. We consider this level to be 25% of their maximum volitional isometric contraction force (MVIC).

3.5.2.2 Training Period

Subjects then commenced the actual training period using NMES. This consisted of 5 sessions per week (Monday to Friday) for 6 weeks with each session 20 minutes in duration. Participants were instructed to use the device early in the morning between 8am and 10 am to minimise muscle fatigue that may occur after normal daily activities. Subjects were encouraged to increase stimulation intensity within and between each session during the training period.

3.5.2.3 Compliance

A log diary was provided to all subjects in the NMES group to record session date, duration and stimulation intensity during both conditioning and training periods (Appendix III). The stimulator has a built-in log that records total treatment time, and average stimulation intensity used for the previous 4 sessions. Subjects were not informed of this facility. Given that the device does not function unless attached to a subjects' thigh, this enabled comparison between patient reported and device recorded compliance. Readings from both the device and logbook were documented at weeks 2, 5 and 8 of the training program.

3.5.3 Control Group

Subjects assigned to the control group received standard preoperative care. This included advice from a qualified physiotherapist on preoperative range of motion and strengthening exercises. Adherence to this program was not recorded, thereby reflecting normal care in this cohort.

3.6 DETERMINATION OF MUSCLE STRENGTH

Isokinetic force is related to angular velocity with less compressive forces required at higher speeds. Deficits usually appear more pronounced at slower speeds. It is a safe method of assessment as an individual will never meet more resistance than they can tolerate. This is because the resistance is equal to the force applied.

A Biodex Multi-joint System-3 dynamometer (Biodex Medical Instruments, Shirley, NY) was used to assess muscle strength (peak torque) of both the involved and uninvolved limbs (Figure 3.6). Isokinetic quadriceps and hamstring strength were first determined at two angular velocities (60°/sec and 120°/sec). Maximum volitional isometric contraction force (MVIC) and maximum tolerated electrically induced contraction force (MTIC) were then determined for the quadriceps femoris only. This allowed us to assess the tolerance of subjects to NMES in the intervention group and to calculate if they were training at sufficient intensities necessary for a strengthening program (MTIC > 25% MVIC).

The dynamometer samples torque data which is transferred to a second computer for processing using Biodex Advantage Software (Version 3.30, Biodex Medical Systems, Shirley, New York). This permits graphical visualisation on the monitor during testing. There was a 10 minute rest period between each test (isokinetic, MVIC and MTIC) to minimise muscle fatigue. All assessments were performed by a single research assistant blinded to group assignment. Standardised verbal instruction was given during each test.

3.6.1 Subject Positioning and Set-up

Patients were not permitted to visualise the monitor during testing as recommended by the manufacturer. Subjects were positioned in accordance with previous studies.(255;256) The dynamometer was oriented at 90° with 0° tilt. A hip angle of 110° was achieved by inclining the seatback to 70°. This ensures an optimal length-tension relationship of both hamstring and quadriceps muscle groups resulting in improved muscle output. The lower

portion of the ankle force pad was positioned 5 cm above the medial malleolus and the fulcrum of the dynamometer lever arm, containing a force transducer, lined-up with the inferior aspect of the lateral epicondyle. To ensure correct saggital alignment between the tibiofemoral joint and the dynamometers' axis of rotation, the knee was extended from 90° to 0°. Correct axis alignment avoids stressful joint loading and avoids recruitment of other muscles.

Before testing, gravity correction was performed with the knee at 30° flexion. This is necessary since the QFM must overcome the weight of the leg before force is registered. In addition a carpenter's level was used to ensure the lever arm of the dynamometer was vertical with the knee flexed to 90 degrees. Waist, thigh, and shoulder straps stabilised subjects during testing and both arms were placed across their chest (Figure 3.6). These methods were employed to minimise the effects of accessory muscles during knee flexion and extension.

Figure 3.6. Dynamometer setup

3.6.2 Isokinetic Quadriceps Femoris and Hamstring Strength Assessment

Three sub-maximal warm-up repetitions and one maximal repetition were performed to familiarise subjects with the testing protocol. This was immediately followed by five maximal concentric isokinetic repetitions performed at 60 and 120 degrees per second. A rest

period of 60 seconds was given between testing at the two speeds. A set of repetitions was repeated if a correlation coefficient of more than 15% was observed in either quadriceps or hamstrings strength tests. While we aimed for a motion arc of 80° this was not possible in some subjects due to a fixed flexion deformity of the knee. The maximum force (peak torque) determined was recorded for later analysis.

3.6.3 Maximum Volitional Isometric Contraction Force (MVIC)

Isometric peak torque was tested with the knee at 60° of flexion. Once again subjects performed three sub-maximal and one maximal contraction to familiarise themselves with the different procedure. The testing protocol consisted of three consecutive 5 second trials of knee extension with the knee held in 60° flexion. There was a rest period of 50 seconds between each attempt, and a set repeated should the coefficient of variation be greater than 15%. The maximum force generated over the three trials was recorded as the peak torque.

3.6.4 Maximum Tolerated Induced Contraction Force (MTIC)

There was a ten minute rest period during which the subject was prepared for the MTIC test. To minimise impedance, both thighs were cleaned with an alcohol wipe ensuring good contact between the skin and the electrodes. Side specific NMES units (KneeHab-II) were securely fitted and the subject repositioned on the dynamometer. The stimulator has a rechargeable battery which was fully charged prior to each testing session. Furthermore, a new set of electrodes were used for each testing session on each subject.

A series of brief electrically stimulated contractions lasting less than 5 seconds were provided with increasing intensity (at 5 unit intervals) until the maximally tolerated stimulation level was achieved for each subject. This was repeated an additional two times to determine the maximum intensity tolerated by each subject.(256) There was a further rest period of five minutes to allow for muscle fatigue.

To determine the MTIC, five consecutive electrically stimulated contractions lasting 5 seconds (excluding 1.5 seconds ramp-up time and 0.5 seconds ramp down time) were elicited with a rest of 50 seconds between each. Subjects were instructed to relax and not contribute any volitional effort during the electrically stimulated contraction: "relax and let the stimulator do the work". The peak force generated by the stimulator was recorded as the MTIC. The percentage of MVIC produced by the electrically induced contraction was calculated with the formula:

$$\%MVIC = (MTIC/MVIC) \times 100$$

3.7 MUSCLE CROSS-SECTIONAL AREA

A Gyroscan Intera 1.5T magnetic resonance imaging (MRI) scanner (Philips Medical Systems, Holland) was used to determined QFM cross-sectional area (CSA) of both thighs using a 4 mm slice thickness: 0.4 mm slice gap, 100 ms echo time; and 3000 ms relaxation time. This modality has been used by several researchers to calculate QFM CSA.(12;48;257) While scanning the entire muscle length is considered the gold-standard for determining quadriceps volume, the anatomical cross-sectional area has been shown to estimate muscle volume with a 10% error using regression analysis.(258)

Patients were placed supine with a surface body coil placed around both thighs. A coronal scouting scan established the level of the mid-thigh using the greater trochanter and lateral knee joint line as anatomical markers. A total of twelve T2-weighted axial images were produced with a field of view of 30 cm (256 x 256 pixel matrix). Images were transferred to a second computer for analysis using a preinstalled image software package (Scion Image for Windows, ver4.0.3.2, Scion Corp, Frederick, MD, USA). After spatial calibration was performed a single clinician, blinded to group assignment, used manual planimetry to outline the QFM as the region of interest (Figure 3.7), with QFM CSA (cm^2) automatically calculated. The average area of the central two images was recorded as each subjects' QFM CSA.

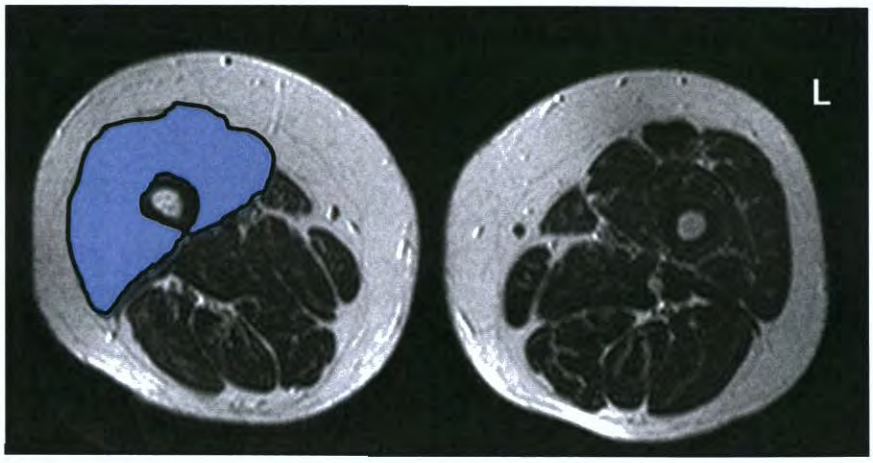


Figure 3.7. Determination of quadriceps femoris CSA from MRI scans

3.8 ANTHROPOMETRIC EVALUATION

Detailed measurement of knee range of motion (ROM, degrees) and extension lag (degrees) were taken using a goniometer (Figure 3.8).(259;260) Thigh circumference (cm) was assessed at the mid-point between the greater trochanter and the lateral joint line to correspond with MRI CSA evaluation. A physiotherapist, blinded to subject group assignment, performed the assessments. Height (m) and weight (kg) were measured with the subject barefoot and wearing shorts and a light top. Body mass index (BMI, kg/m^2) was calculated accordingly.

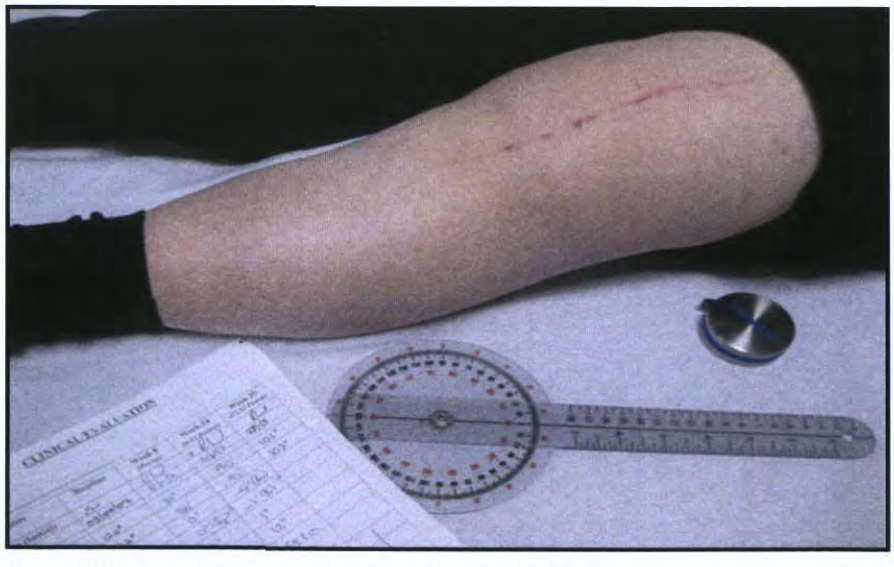


Figure 3.8. Equipment for anthropometric evaluation

3.9 OBJECTIVE FUNCTIONAL CAPACITY

A timed chair-rise test (TCT), a 25-metre timed walk test (TWT), and a timed stair climb test (TST) were used to assess functional capacity.(247) Each test was performed 3 times and the fastest used for analysis. Subjects were allowed a practice attempt for each test to familiarise themselves with the respective protocol. All assessments were performed single blind in the same order for each participant.

3.9.1 Timed Chair-Rise Test

A straight back chair with adjustable leg height was used for the timed chair-rise test (TCT). This permitted a standardised sitting position with each subjects' knees flexed to 90° and their feet flat on the ground. The test was performed with the arms folded across their chest. Subjects were permitted to perform the test using the armrests if required. Each test consisted of three cycles of standing up from a seated position and then sitting back down again in rapid succession (stand-sit-stand-sit-stand-sit). The total time taken was recorded. Subjects were instructed to "stand up fully and sit down three times as quickly and as safely as you can".

Figure 3.9. Adjustable chair for timed chair-rise test

Figure 3.11. Stairwell used for timed stair-climb test

3.10 SELF-REPORT OUTCOME MEASURES

We used validated self-administered scoring questionnaires to evaluate subjective perception of disability (Appendix IV). It has been proposed that self-report measures relate to knee pain and strength whereas performance (functional) measures relate to self-efficacy; thus both examine different aspects of mobility.(262)

3.10.1 Western Ontario McMaster University Universities Arthritis index (WOMAC)

The WOMAC is a validated questionnaire designed for subjects with hip or knee osteoarthritis and assesses the patient's perception of their disability.(263) It consists of 24 questions scored from 0 to 4 (best to worst respectively). These are sub-categorized into pain (0-20), stiffness (0-8), and function (0-68).

3.10.2 Short Form-36 (SF-36)

The Medical Outcomes Study 36-Item Short Form Health Survey (SF-36) was formed as a culmination of several scales in the Medical Outcomes Study (MOS).(264) It evaluates general health using a 36 item questionnaire under 8 parameters: physical and social functioning, role limitations because of emotional problems or physical problems, mental health, bodily pain, vitality, and general health perceptions. Component scores are given for

physical and mental health, both scored from 0 to 100 with a higher score indicating better health. Reliability and validity have been established.(265-267)

3.10.3 Oxford Knee Score

The Oxford knee score is a validated 12 item questionnaire designed to assess the impact of knee pain on activities of daily living during the previous 4 weeks in patients with knee osteoarthritis.(268) Each item is scored on a 5 point likert scale to provide summated scores from 12 to 60 (best to worst respectively).

3.11 PERCUTANEOUS MUSCLE BIOPSY

Samples were obtained from the *m vastus lateralis* one week before baseline (week -1) and at 48 hours after completion of the program (week 8) . No biopsies were performed after surgery to avoid the risk of implant infection. The justification for obtaining the first sample one week before the other baseline assessments was to allow for testing and training to be performed after the wound had healed. It also permitted wound review and a delay of further assessment should any wound complications arise.

The first sample was obtained under local anaesthetic using 5mls 1% w/v lidocaine HCl infiltrated into the skin, adipose tissue and muscle fascia. The second was taken immediately before the limb had been exsanguinated prior to surgery (TKA), and was performed under epidural anaesthesia. In addition, the second sample was taken 1cm distally to the previous biopsy site to avoid the inclusion of scar tissue.

3.11.1 Muscle Biopsy Procedure

About 100mg of muscle was obtained from the *m. vastus lateralis* of the affected limb using the percutaneous needle biopsy technique described by Bergstrom with the aid of suction.(269) The site for the biopsy was midway between the upper pole of the patella and the anterior superior iliac spine at the anterior border of the iliotibial band. Lying supine, subjects contracted their thigh to help determine this position which was then marked with an

indelible pen. The region was shaved and cleaned with an alcohol swab. The biopsy area was further cleansed using povidine-iodine solution and a sterile drape placed around the incision site.

A small incision (0.5cm) was made through the skin and subcutaneous fat using a number 11 scalpel and the fascial fibres separated with the blunt edge of the blade. Subjects were warned they would experience slight pressure as the 5-mm biopsy needle (Figure 3.12) was inserted through the incision into the muscle. A 200ml syringe attached to the proximal port of the needle permitted suction to be applied to draw the sample into the needle port. Closure of the port using a sharp trocar completed the acquisition of the sample. The biopsy sample (approximately 50mg) was collected and the procedure repeated to obtain a total of 100mg muscle. Samples were snap-frozen in liquid nitrogen and stored at -80° C until analysis.

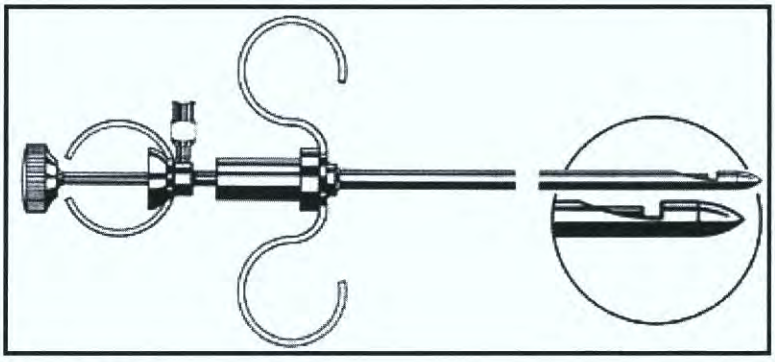


Figure 3.12. Bergstrom needle

After the procedure, direct pressure was applied to minimise haematoma formation. The incision was closed with steri-strips and the wound covered by an adhesive dressing. Written instruction for post-biopsy care was provided to each subject (Appendix VI). The wound was reviewed one week after the first biopsy by a clinician.

3.11.2 Complications of Muscle Biopsy

Complications accompanying this procedure are rare. The primary concern would be prolonged bleeding which could produce a bruise in the area. This may cause some muscle soreness, but is adequately treated with rest, ice, compression, and elevation. Although *m.*

vastus lateralis has no major vessels or nerves in the areas where the biopsy was performed, some small cutaneous nerve branches can be damaged causing temporary symptoms (numbness or paraesthesia). Care was taken to employ standard precautions to avoid infection, including the "universal precautions" for the handling of blood and infectious materials.

3.12 MUSCLE SAMPLE ANALYSIS

All biopsy samples were stored for later analysis in Dublin City University at -80° C. They were prepared in batch and analysed simultaneously to minimise systematic error.

3.12.1 RNA Isolation

Total RNA was isolated from 25-30 mg crude muscle tissue based on the acid guanidinium thiocyanate-phenol-chloroform extraction method of Chomczynski & Sacchi (270) using TRI reagent (Sigma-Aldrich, UK; T9424) as per the manufacturer's instructions (Molecular Research Centre, Cincinnati, OH; accessed at www.ambion.com/techlib/prot/bp_9738.pdf).

Figure 3.13-a.

Reagents used for RNA extraction:

A: Chloroform – Extract RNA

B: Isopropanolol – Precipitate RNA

C: Ethanol – Wash resulting RNA pellet

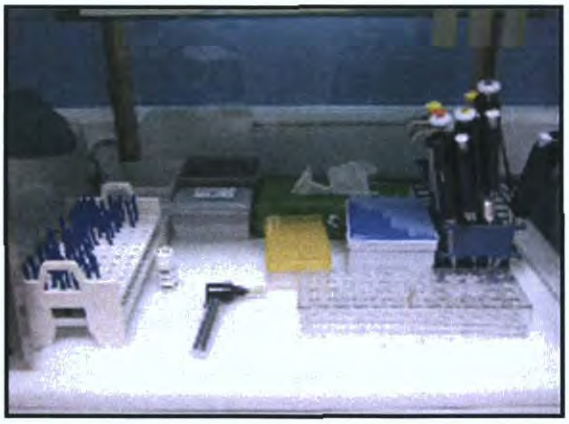


Figure 3.13-b.

Equipment used for RNA extraction

PROCEDURE FOR RNA EXTRACTION:

1. Muscle tissue was lysed in a 1.5 ml eppendorf (Figure 3.14) using a motorised pestle in 400 µl of TRI reagent (4° C) per 50 mg of tissue, followed by drawing through a 25G needle with a 1 ml syringe.

Figure 3.14. Muscle sample in TRI reagent

2. Samples were then centrifuged at 11310 x g for 10 minutes at 4° C producing a
    - Supernatent – RNA, DNA, & Protein
    - Pellet – Soft Tissue

Figure 3.15. Centrifuge

3. The supernatant was transferred to a fresh tube and 80 µl of Chloroform added. The samples were mixed vigorously and then left to incubate for 15 minutes
4. Samples were centrifuged again at 11310 x g for 15 minutes at 4° C producing 3 distinct layers (Figure 3.16):
    - Upper aqueous phase – Total RNA (clear)
    - Middle phase – DNA (cloudy)
    - Bottom Phase – Protein (Pink)

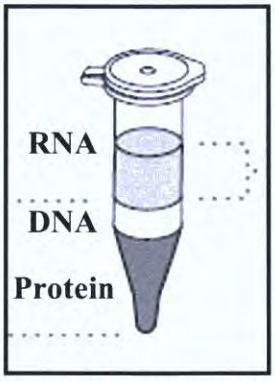


Figure 3.16. RNA isolation

5. The aqueous phase containing RNA was transferred to a fresh tube and 200 µl Isopropanolol added to precipitate the RNA. This was vortex mixed, left to incubate for 10 mins at room temperature and then centrifuged at 11310 x g for 10 minutes at 4° C.
6. The supernatant was discarded and an RNA pellet remained at the bottom of the eppendorf (Figure 3.17).

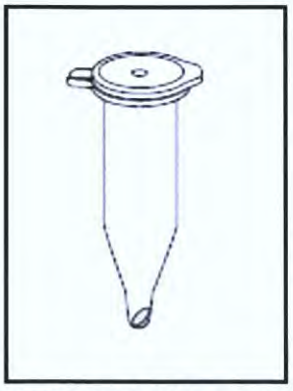


Figure 3.17. RNA pellet

7. 75% Ethanol (400 µl) was added and vortex mixed to wash the pellet. This was centrifuged again for 5 minutes at 11310 x g at 4° C. The ethanol was removed and the pellet air dried for 10 minutes.

8. RNA samples were redissolved in 30 µl RNAse free water (Figure 3.18) and stored at -80° C.

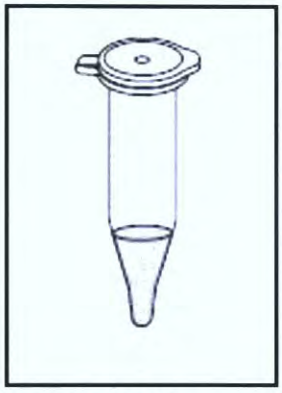


Figure 3.18. RNA solubisation

3.12.2 RNA Quantification

Total RNA concentration (ng/µl) was determined spectrophotometrically at an absorbance of 260 nm and the integrity of each RNA sample verified by gel electrophoresis (RNA 6000 Nano Lab Chip & 2100 Bioanalyzer, Agilent Technologies, Palo Alto, CA; accessed at www.ambion.com/techlib/prot/bp_9738.pdf) and by measuring the spectrophotometric 260/280 nm ratio (>1.8).

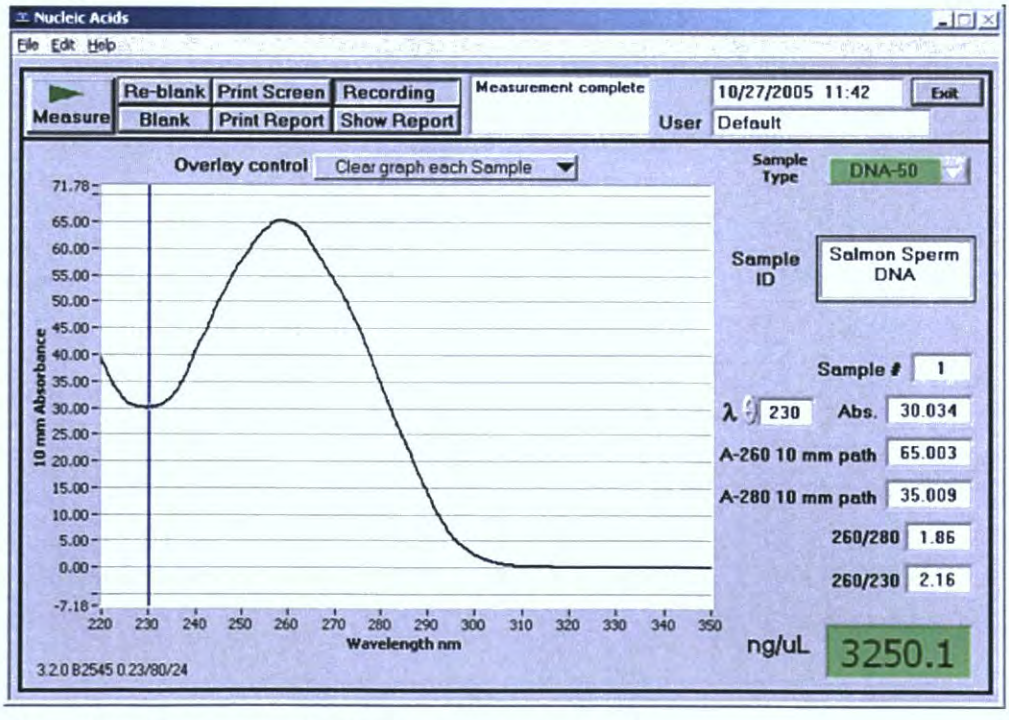


Figure 3.19. Nanodrop spectrophotometer sample output

3.12.3 Reverse Transcription to cDNA

RNA from each sample was reverse transcribed to cDNA using the Reverse Transcription system (A3500; Promega, Madison, WI) primed with oligo-$dT_{(15)}$ (random hexamer) as per the manufacturer's instructions (TB-099, Applied Biosystems, Foster City, CA).

A master mix was made by combining 160 µl $MgCl_2$ [25mM], 80 µl TaqMan RT buffer [10X], 80 µl dNTP mixture [10mM], 20 µl RNase inhibitor [20U/µl], 24 µl Reverse Transcriptase [25U/µl], and 40 µl random hexamer [50U/µl]. One microgram of mRNA was added to 10.1 µl of this master mix and then RNAse free water added to make a total volume of 20 µl. The thermal cycling parameters were used in the order as outlined in table 3.2 to complete the process. The cDNA template was then stored at -20° C until subsequent analysis.

Table 3.2. Thermal cycling parameters for reverse transcription to cDNA

Stage	Process	Temperature	Duration
1	Incubation	25° C	10 mins
2	Reverse Transcription	42° C	60 mins
3	Inactivation	90° C	5 mins

3.12.4 Quantitative Real-Time PCR

qRT-PCR was performed using the ABI Prism 7500 Sequence Detection System and software package (version 1.1; Applied Biosystems, Foster City, CA) using Assay-on-Demand pre-designed gene-specific primer and probe sequences (P/N 4331182; Taqman® Gene Expression Assays, Applied Biosystems, Foster City, CA). The PCR reaction mix in each well consisted of 30 ng cDNA template (5 µl; [6 ng/µl]), 1 µl Taqman probe with forward and reverse primer set, 10 µl Taqman Universal Master Mix (Applied Biosystems, Foster City, CA) and 4 µl nuclease-free water in a 20 µl reaction. Gene targets for RT-PCR are reported in table 3.3.

Table 3.3. Gene targets for qRT-PCR

Common Name	Target	Gene ID	TaqMan Assay ID
MHC-I	MYH7	4625	Hs01110632_m1
MHC-IIa	MYH2	4620	Hs00430042_m1
MHC-IIx	MYH1	4619	Hs00428600_m1
IGF-1	IGF1	3479	Hs01547656_m1
MURF-1	TRIM63	84676	Hs00822397_m1
MAFbx	FBXO32	114907	Hs01041408_m1

Assay ID corresponding to the TaqMan® Gene Expression Assay ID (P/N 4331182; Applied Biosystems)

The PCR profile for all genes consisted of one cycle at 50°C for 2 min, followed by a denaturing cycle at 95°C for 10 min, followed by 40 cycles of denaturing at 95°C for 15 sec and annealing and elongation at 60°C for 1 min. Each sample was run in duplicate. mRNA content was calculated from a corresponding standard curve (critical threshold cycle number vs. log dilution) run together with the samples. The standard curve was constructed using serial dilutions of an RNA sample pooled from the entire sample set and included in the RT-PCR reaction. The average critical threshold cycle (C_T) value of the unknown samples was converted to relative expression data using the appropriate standard curve. mRNA data is expressed as the ratio between the gene of interest and a housekeeper gene, allowing normalisation of mRNA expression to a stable mRNA.

The selection of a housekeeping gene as an endogenous control requires several considerations: (i) expression of the gene must stay constant throughout the given intervention; (ii) amplification efficiency of the gene should be similar to that of the gene of interest; (iii) abundance of housekeeping gene should be similar to that of genes of interest. In general, housekeeping genes should be constitutively expressed and minimally regulated. We selected GAPDH (94333764F, Applied Biosystems, Foster City, CA) as the endogenous control to determine relative expression of the target mRNA.

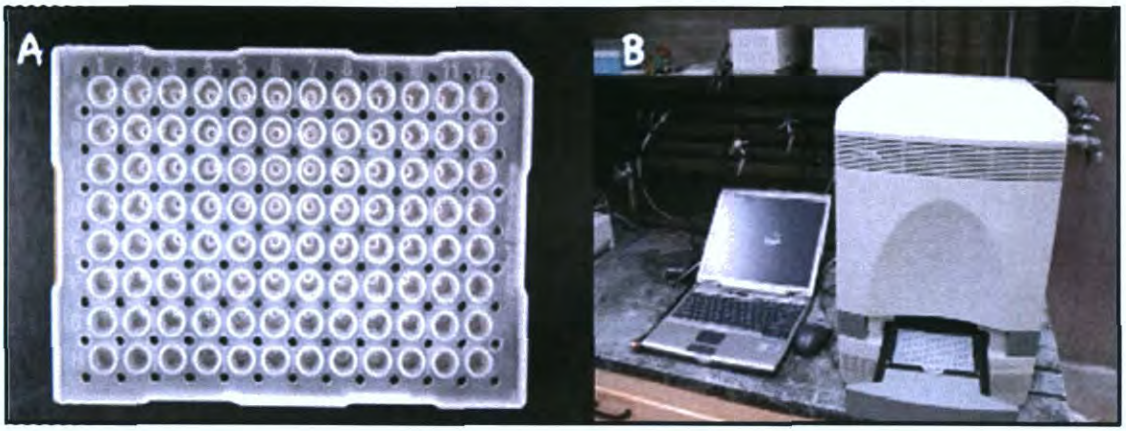


Figure 3.20.

Panal A: Sample of a plate used for qPCR. **Panal B:** ABI Prism 7500 sequence detection system

3.13 STATISTICAL ANALYSIS

Independent t-tests were used to evaluate potential group differences for age, height, weight, and walking time. The non-parametric Friedman test was used to compare within group differences in strength, CSA, objective function, clinical evaluation, and subjective outcome. Post hoc analyses were performed using the Wilcoxan signed ranks tests with Bonferroni adjustment for multiple comparisons. Between group comparisons were performed using the Mann Whitney test. The relation between selected parameters was determined using Spearman rho correlations. Statistical significance was set at p=0.05. Statistical tests were performed using the Statistical Package for Social Sciences version 15.0 (SPSS, Inc., Chicago, IL). Error bars unless stated otherwise show standard errors. Where error bars are merged, it can be assumed that no significant differences exist between groups. Significant differences have been highlighted on the graphs.

Further details of the experimental design and procedures specific for each study are described in the respective Chapters VI, V, and VI.

Chapter IV

Quadriceps Femoris Neuromuscular Electrical Stimulation in Knee Osteoarthritis: Effects on Muscle Strength and Clinical Function

4.1 INTRODUCTION

Osteoarthritis (OA) is considered the primary cause of chronic disability in adults over 65 years.(145;171) The Framingham Osteoarthritis Study reported higher rates of knee OA with increasing age, observing radiographic disease in 27% of adults below 70 years of age compared to 44% over 80 years.(144) European levels may not be as high with recent data from Spain citing rates of 9% and 15% for elderly men and women respectively.(271)

There is increasing evidence that quadriceps muscle dysfunction is a causative factor in the development of knee OA and may potentiate disease progression.(149;156) A study of muscle function following arthroscopic meniscectomy found weakness was specific to the quadriceps femoris muscle (QFM) with sparing of the hamstring and adductor muscle groups.(157) A longitudinal study, however, found no association between QFM strength and clinical deterioration despite radiological disease progression.(158) This disparity may be methodological since some studies determined disease severity arthroscopically while others used radiological measures. The association between plain film radiology and clinical symptoms is often poor.(159) Individuals with QFM weakness have an associated limitation in performing normal functional tasks such as walking, rising from a chair and climbing stairs.(96;164) This impairment is also reflected by poorer subjective functional scores.(149;159)

In populations with knee OA, supervised exercise training programs can increase muscle strength and improve function as well as reduce self-reported knee pain.(186;272) However, such programs are considered complex, expensive and labour intensive.(161;273) Strength improvements found with unsupervised home-based exercise program are less than

occurs with supervised training.(197) Explanations for this difference include training at lower intensities and poor program adherence.

Muscle activation failure is the principle mechanism potentiating quadriceps weakness in end-stage knee OA and concern has been raised regarding the efficacy of exercise programs in subjects with advanced disease.(154;166) Volitional exercise may not increase muscle strength if patients cannot overcome these activation deficits.(274) Therapies that can reverse sensorimotor dysfunction could play a therapeutic role in subjects with knee OA.(275)

Neuromuscular electrical stimulation (NMES) induces involuntary muscle contraction by stimulating the underlying neuromuscular junction and surrounding muscle fibres. Larger motor units (type II fibres) are recruited to a greater extent than normally occurs during volitional activity.(240) NMES results in the contraction of fibres that are not otherwise activated in subjects with knee OA, providing an alternative strengthening modality. Recent studies have shown NMES to significantly improve strength and function in subjects with early knee OA, with one citing it to be as effective as exercise training.(247;248) To date, no studies have assessed the effect of NMES on strength and functional capacity in subjects with advanced knee OA.

The primary aim of this study was to determine the efficacy of a home-based NMES program on muscle strength and objective functional capacity in a cohort with end-stage knee OA. In addition, determinants of self-efficacy and pain were evaluated as well as changes in subject tolerance to stimulation intensity.

4.2 PATIENTS AND METHODS

4.2.1 Patients

Men and women aged between 45 and 80 years undergoing TKA for advanced primary knee OA were recruited from a preoperative orthopaedic assessment clinic between July and October 2007. Block randomisation was used to allocate subjects to either intervention (NMES) or control (Con) groups. Approval for this study was given by the local ethics review committee. Patients received individual counselling to ensure they understood the full nature of the study before informed consent was obtained. Seventeen patients enrolled in the study. One control subject withdrew due to marked deterioration in her preoperative clinical condition. Baseline demographics for the 16 patients (NMES=10; Con=6) who completed the study are presented in table 4.1. There was no difference between groups in any of the measures parameters.

Table 4.1. Baseline characteristics

	Group		
	NMES	**Control**	**p value**
Male: Female	4 : 6	2: 4	
Age (y)	64.6 ± 7.6	64.8 ± 11.0	0.960
Height (cm)	162.4 ± 13.3	158.0 ± 7.2	0.471
Weight (kg)	81.1 ± 13.1	79.1 ± 13.1	0.767
BMI (kg/m^2)	30.7 ± 2.9	31.8 ± 6.1	0.616
Walking Dsitance (m)	1815.0 ± 2222.1	958.3 ± 801.5	0.384
Walking Time (min)	22.8 ± 22.6	17.0 ± 10.4	0.333

Values are means ± SD; BMI: Body Mass Index

4.2.2 Overview

The intervention group received 8 weeks unsupervised, home-based NMES training applied unilaterally to the QFM of the affected side. Subjects assigned to the control group received standard preoperative care including advice from a physiotherapist on range of motion and strengthening exercises. Muscle strength and quadriceps maximum tolerated electrically induced contraction force (MTIC) were determined at baseline, week 2, 5 and 8. Functional and anthropometric assessments were performed and questionnaires administered at baseline and at the end of the study period.

4.2.3 Intervention – Neuromuscular Electrical Stimulation

The stimulator (KneeHAB II, Bio-Medical Research, Galway, Ireland) was used for 20 min·day^{-1} on alternate days during the initial two week conditioning period. The training program involved a single 20 min session·day^{-1}, 5 days·wk^{-1} for 6 weeks. Patients were encouraged to increase stimulation intensity to their maximum tolerated level at an early stage during each session so that they would train at their highest tolerated intensity for as long as possible. When the stimulation was perceived to be painful, subjects were advised to reduce stimulation to a tolerable level and to then gradually increase it again if time remained.

4.2.4 Compliance and Training Intensity

Although the device automatically turned-off after completion of each 20 min session, subjects recorded session duration in their logbook and, when applicable, the reason for cutting-short a training session. Subjects also recorded the maximum training intensity they achieved (up to a maximum of 99 units) during each training session. In addition and unknown to the patients, the stimulator recorded total usage and the average training intensity for the last four training sessions. The information from the stimulator was obtained when subjects attended for strength evaluation at weeks 2, 5 and 8.

4.2.5 Tolerance and %MVIC

The MTIC of the involved and uninvolved QFM was determined for both the NMES and Con groups as outlined previously (Chapter III). The maximum stimulation level tolerated by each subject was recorded (up to a maximum intensity of 99 units), and was considered to reflect tolerance. The peak torque of the electrically induced contraction was calculated as a percentage of the corresponding maximum voluntary isometric contraction force (MVIC) for each limb [%MVIC = (MTIC/MVIC) x 100]. Subjects assigned to the NMES group were informed of their maximum stimulation level achieved at baseline, week 2 and week 5 assessments and were instructed to train at these intensities.

4.2.6 Evaluation Protocols

4.2.6.1 Muscle Strength

Hamstring (HS) and quadriceps (QFM) muscle strength of the involved and uninvolved limbs wee determined as outlined in chapter III. Concentric isokinetic QFM (extension) and HS (flexion) peak torque (Nm) were assessed at 60°/sec and 120°/sec. QFM MVIC peak torque (Nm) was assessed with the knee flexed at 60°. The highest force generated in each test was recorded as the peak torque. Standardised verbal coaching was given by a research assistant blinded to each participant's group assignment.

4.2.6.2 Functional and Anthropometric Evaluation

A timed chair-rise test (TCT), 25-metre timed walking test (TWT), and a timed stair climbing test (TST) were used to assess functional capacity. Each test was performed 3 times with the fastest time used for analysis. All assessments were performed in the same order (TCT → TWT → TST) for each participant. A goniometer was used to determine knee range of motion (ROM) and extension lag (degrees). Thigh circumference (cm) was assessed at the mid-point between the greater trochanter and the lateral joint line.

4.2.6.3 Self-Report Outcome Measures

Three subjective scoring instruments were used to assess individual perception of disability. The Western Ontario McMaster Osteoarthritis Index (WOMAC) can be categorized into pain (0-20), stiffness (0-8), and function (0-68) with a lower score in each indicating a more favourable perception of disability. The Medical Outcome Study short form 36 (SF-36) evaluated general health with component scores for physical and mental health. Both components are scored from 0 to 100 with a higher score indicating better health. The Oxford knee score is a 12 item questionnaire that uses a 5 point likert scale to give summated scores of 12 to 60 (best to worst respectively).

4.2.7 Statistical Analysis

Independent t-tests were used to evaluate group differences at baseline. The Friedman test compared within group differences in strength, function, anthropometric measures and subjective outcomes. Post hoc analyses was performed using the Wilcoxan signed ranks tests with Bonferroni adjustment for multiple comparisons. Between group comparisons were performed using the Mann Whitney test. The relation between selected parameters was determined using Spearman rho correlations. Statistical significance was set at $p=0.05$. Statistical tests were performed using the Statistical Package for Social Sciences version 15.0 (SPSS, Inc., Chicago, IL).

4.3 RESULTS

4.3.1 Compliance and Training Intensity

Overall patient reported compliance (logbook) was 99.5% (range, 97.1 to 100%), and overall stimulator recorded compliance was 96.1% (range, 69.7 to 114.8) (Table 4.2). There was much variability in compliance recorded by the stimulator which was equal to or more than 100% in all subjects during the 2 wk conditioning period. From wk 5 to wk 8 stimulator recorded compliance reduced to 92%. Subjects reported a compliance rate of approximately 99% over the same period.

Table 4.2. Compliance and training intensity

	Time		
	Week 2	**Week 5**	**Week 8**
Compliance			
Logbook	99.9 ± 0.4	100.0 ± 0.0	98.5 ± 3.4
Stimulator	103.5 ± 9.3	99.5 ± 12.2	91.6 ± 18.0
Training Intensity			
Logbook (Maximum)	90.3 ± 9.2	97.9 ± 2.9	99.0 ± 0.0
Stimulator (Average)	83.9 ± 8.5	93.4 ± 6.2 [a]	94.9 ± 4.5 [a]

Logbook training intensity is the maximum training intensity reported by subjects. Stimulator training intensity is the average stimulation intensity used by subjects over the previous four training sessions.; Values are means ± SD; [a] $p<0.05$ vs. Week 2

The maximum training intensity achieved by subjects after the initial two wk conditioning period was 90.3 units (range, 75 to 99) with four subjects tolerating the devices' maximum stimulation intensity (Table 4.2). At wk 5, eight subjects tolerated maximum stimulation intensity and by wk 8, all subjects reported training at the maximum intensity level. Compared to wk 2, the average training intensities recorded by the stimulator were greater at wk 5 ($p=0.008$) and wk 8 ($p=0.007$). There was no difference in average stimulation levels recorded by the stimulator from wk 5 to wk 8 ($p=0.635$).

4.3.2 Tolerance and %MVIC

Maximum stimulation intensity, MTIC, MVIC and %MVIC were similar in both groups at baseline (Tables 4.3). Overall, both groups tolerated higher stimulation intensities in both limbs at wk 8 compared to baseline although the increases were not statistically significant. The NMES group tolerated higher stimulation intensities in the involved (trained) limb compared to the control group at wk 2 (p=0.046), wk 5 (p=0.05), and wk 8 (p=0.017).

Table 4.3. Tolerance, MTIC and %MVIC of the involved and uninvolved limbs

	Time			
	Baseline	**Week 2**	**Week 5**	**Week 8**
INVOLVED LIMB				
Maximum Stimulation Intensity (Units)				
NMES	91.0 ± 10.4	97.7 ± 3.0 †	98.6 ± 1.3 †	99.0 ± 0.00 †
Control	82.8 ± 13.3	91.3 ± 7.8	92.8 ± 7.7	92.8 ± 7.7
MVIC (Nm)				
NMES	87.4 ± 36.9	101.4 ± 45.4	106.0 ± 46.8 a	110.0 ± 56.1
Control	92.2 ± 31.2	103.1 ± 30.1	106.6 ± 30.8	105.5 ± 30.7
MTIC (Nm)				
NMES	30.7 ± 23.6	33.7 ± 30.0	36.7 ± 22.8	36.3 ± 22.7
Control	25.4 ± 19.1	35.1 ± 23.3	32.5 ± 22.0	37.7 ± 24.7
%MVIC (%)				
NMES	33.7 ± 17.0	32.9 ± 22.2	34.6 ± 15.3	35.4 ± 17.3
Control	26.0 ± 14.8	31.5 ± 16.1	28.7 ± 15.7	35.5 ± 25.0
UNINVOLVED LIMB				
Maximum Stimulation Intensity (Units)				
NMES	90.5 ± 11.1	95.4 ± 5.7	96.3 ± 6.0	97.7 ± 3.0
Control	81.5 ± 14.1	91.3 ± 11.0	94.3 ± 9.6	95.0 ± 9.8
MVIC (Nm)				
NMES	112.0 ± 54.5	119.2 ± 59.6	121.5 ± 58.3	130.6 ± 66.1
Control	89.6 ± 29.3	110.9 ± 23.2	111.6 ± 26.6	103.5 ± 25.1
MTIC (Nm)				
NMES	35.7 ± 25.4	43.0 ± 31.4	51.1 ± 36.3	45.8 ± 34.2
Control	18.9 ± 13.0	28.9 ± 17.1	31.6 ± 18.9	33.6 ± 22.9
%MVIC Nm)				
NMES	32.2 ± 16.6	39.5 ± 24.5	43.2 ± 22.6	37.8 ± 23.2
Control	22.5 ± 14.1	26.9 ± 16.5	30.6 ± 21.9	35.1 ± 28.0

MVIC – Maximum Voluntary Isometric Contraction; MTIC – Maximum Tolerated Induced Contraction
%MVIC – MTIC peak torque expressed as a percentage of MVIC peak torque
Values are means ± SD; [a] p<0.05 vs. baseline; † p<0.05 vs. Control

There was a significant inverse relation between body mass index (BMI) and maximum stimulation intensity tolerated in the involved limb at wk 2 (r^2=0.20; p=0.042), wk 5 (r^2=0.26; p=0.021), and wk 8 (r^2=0.20; p=0.041). There was no correlation between BMI and uninvolved limb tolerance.

Quadriceps MTIC and its corresponding %MVIC did not change significantly in either group over the study period (Table 4.3). MTIC of the involved limb was greater than 25% in both groups at all time-points. There was a significant relation between MTIC and MVIC at each time point in both limbs (Table 4.4).

Table 4.4. Significant correlations between MVIC and MTIC

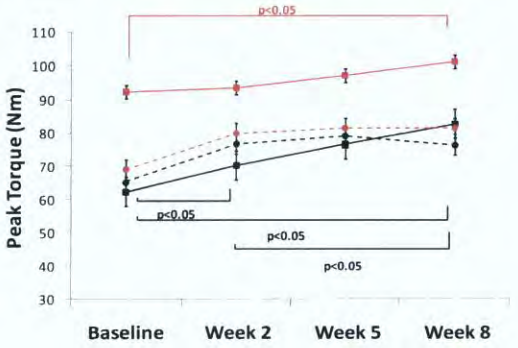
Comparison	p value	r value
Involved Limb		
Baseline	0.004	0.632
Week 2	0.001	0.732
Week 5	0.000	0.745
Week 8	0.031	0.476
Uninvolved Limb		
Baseline	0.006	0.615
Week 2	0.011	0.568
Week 5	0.011	0.564
Week 8	0.029	0.482

p value – significance level; r value – correlation coefficient
MVIC – Maximum Voluntary Isometric Contraction
MTIC – Maximum Tolerated Induced Contraction

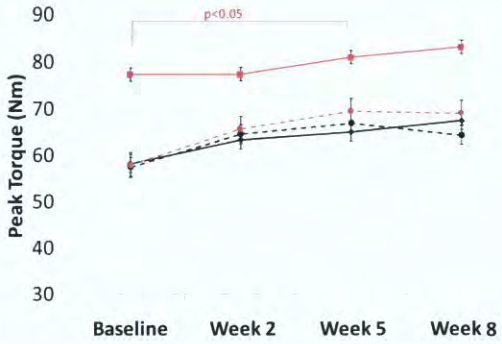
4.3.3 Muscle Strength

There was no difference in muscle strength between groups at any time point during the study (Figure 4.1). QFM and HS strength of both limbs of the Con group did not change significantly over the study period. There was no difference in strength between involved and uninvolved limbs of the Con group during the study period.

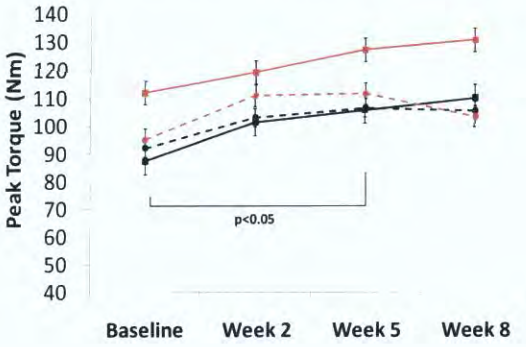
a) Quadriceps isokinetic peak torque – 60°/sec



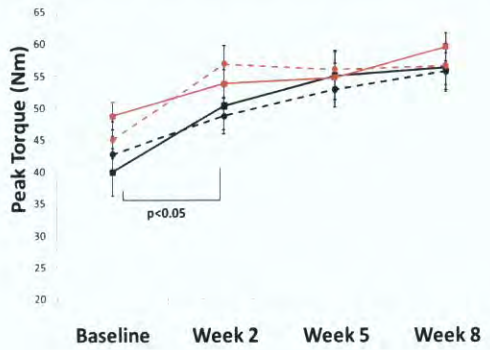
b) Quadriceps isokinetic peak torque – 120°/sec



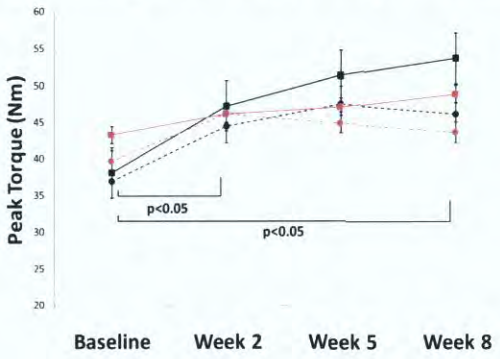
c) Quadriceps isometric peak torque



d) Hamstring isokinetic peak torque – 60°/sec



e) Hamstring isokinetic peak torque – 120°/sec



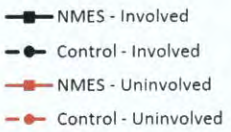


Figure 4.1. Isokinetic and isometric peak torque

Isokinetic QFM peak torque at 60°/sec of the NMES group's involved limb increased at wk 2 (12.6%, p=0.007), wk 5 (22.8%, p=0.009; alpha level, 0.008) and wk 8 (32.8%, p=0.007) compared to baseline. There was no change in involved limb isokinetic QFM peak torque when assessed at 120°/sec. Involved QFM isometric peak torque increased by 21.1% (p=0.007) at wk 5 and 25.9% (p=0.013; alpha level=0.008) at wk 8 compared to baseline. There were smaller increases in uninvolved Isokinetic QFM peak torque at 60°/sec (baseline – wk 8: 9.5%, p=0.007; wk 2 – wk 8: 8.1%, p=0.012; alpha level, 0.008) and at 120°/sec (baseline – wk 5: 4.7%, p=0.007). Uninvolved isometric peak torque was 16.6% greater at wk 8 compared to baseline (p=0.013; alpha level, 0.008).

At baseline, isokinetic QFM peak torque at 60°/sec was 32.4% greater (p=0.009) in the uninvolved limb compared to the involved limb of the NMES group. The uninvolved limb was stronger at wk 2 (24.9%, p=0.022) and wk 5 (21.1%, p=0.047) but at wk 8 there was no significant difference in strength between limbs. Isokinetic QFM peak torque at 120°/sec and isometric peak torque were greater in the uninvolved compared to the involved limb at baseline only by 24.7% (p=0.037) and 22.0% (p=0.047) respectively.

Isokinetic HS peak torque at 60°/sec increased in the involved limb of the NMES group at wk 2 (26.1%, p=0.007), wk 5 (38.0%, p=0.009; alpha level, 0.008) and wk 8 (41.1%, p=0.009; alpha level, 0.008) compared to baseline. At 120°/sec, isokinetic HS peak torque of the involved limb was greater compared to baseline at wk 2 (23.9%, p=0.005), wk 5 (34.6%, p=0.011; alpha level, 0.008) and wk 8 (40.9%, p=0.005). From wk 2 to wk 8, isokinetic HS peak torque at 120°/sec increased by 13.8% (p=0.013; alpha level, 0.008). Isokinetic HS peak torque of the uninvolved limb did not change significantly at 60°/sec. At 120°/sec the increase in isokinetic peak torque from baseline to wk 8 was close to statistical significance (p=0.009; alpha level, 0.008). Uninvolved HS strength was only greater than the involved limb at baseline when assessed at 60°/sec (18.2%; p=0.037).

Baseline body mass index (BMI) was inversely related to QFM isometric strength throughout the study (Table 4.5). Subject height related to HS and QFM strength in both limbs at all time points (Table 4.6). Subject age did not correlate with muscle strength in either limb.

Table 4.5. Significant correlations between muscle strength and subject BMI

Comparison		p value	r value
INVOLVED LIMB			
Quadriceps Isokinetic 60°/sec			
	Week 2	0.050	-0.426
Quadriceps Isokinetic 120°/sec			
	Baseline	0.032	-0.474
	Week 5	0.042	-0.444
	Week 8	0.036	-0.462
Quadriceps MVIC			
	Baseline	0.036	-0.462
	Week 2	0.039	-0.453
	Week 5	0.039	-0.453
	Week 8	0.040	-0.450
Hamstring Isokinetic 120°/sec			
	Week 8	0.032	-0.474
UNINVOLVED LIMB			
Quadriceps Isokinetic 60°/sec			
	Week 2	0.025	-0.926
	Week 5	0.035	-0.934
	Week 8	0.028	-0.943
Quadriceps Isokinetic 120°/sec			
	Week 2	0.030	-0.480
	Week 5	0.042	-0.444
	Week 8	0.048	-0.429
Quadriceps MVIC			
	Baseline	0.021	-0.512
	Week 2	0.015	-0.544
	Week 5	0.033	-0.471
	Week 8	0.019	-0.524
Hamstring Isokinetic 60°/sec			
	Baseline	0.048	-0.429
Hamstring Isokinetic 120°/sec			
	Baseline	0.033	-0.471
	Week 8	0.025	-0.497

p value – significance level; r value – correlation coefficient

Table 4.6. Significant correlations between muscle strength and subject height

Comparison		p value	r value
INVOLVED LIMB			
Quadriceps Isokinetic 60°/sec			
	Baseline	0.005	0.628
	Week 2	0.005	0.628
	Week 5	0.007	0.603
	Week 8	0.012	0.560
Quadriceps Isokinetic 120°/sec			
	Baseline	0.000	0.753
	Week 2	0.003	0.656
	Week 5	0.005	0.618
	Week 8	0.003	0.651
Quadriceps MVIC			
	Baseline	0.007	0.600
	Week 2	0.009	0.584
	Week 5	0.003	0.656
	Week 8	0.001	0.704
Hamstring Isokinetic 60°/sec			
	Baseline	0.004	0.631
	Week 2	0.003	0.663
	Week 5	0.003	0.657
	Week 8	0.002	0.679
Hamstring Isokinetic 120°/sec			
	Baseline	0.001	0.699
	Week 2	0.001	0.725
	Week 5	0.003	0.656
	Week 8	0.003	0.659
INVOLVED LIMB			
Quadriceps Isokinetic 60°/sec			
	Baseline	0.000	0.921
	Week 2	0.000	0.926
	Week 5	0.000	0.934
	Week 8	0.000	0.943
Quadriceps Isokinetic 120°/sec			
	Baseline	0.000	0.902
	Week 2	0.000	0.934
	Week 5	0.000	0.927
	Week 8	0.000	0.961
Quadriceps MVIC			
	Baseline	0.000	0.896
	Week 2	0.000	0.856
	Week 5	0.000	0.877
	Week 8	0.000	0.915
Hamstring Isokinetic 60°/sec			
	Baseline	0.000	0.901
	Week 2	0.000	0.881
	Week 5	0.000	0.795
	Week 8	0.000	0.853
Hamstring Isokinetic 120°/sec			
	Baseline	0.000	0.906
	Week 2	0.000	0.898
	Week 5	0.000	0.793
	Week 8	0.000	0.851

p value – significance level; r value – correlation coefficient

4.3.4 Objective Function

The Con group did not change significantly in any measure from baseline to wk 8 (Figure 4.2) whereas the NMES group improved at all functional assessments: 25 metre timed walk test (TWT) (8.7%, p=0.005), timed stair-climb test (TST) (19.6%, p=0.005), and timed chair-rise test (TCT) (32.5%, p=0.005). Performance of the TCT was 21.9% better (p=0.007) in the NMES group than the Con group at wk 8. There were significant relations found between subject height and all measures of functional capacity at both time points (Table 4.7). Greater BMI was associated with poorer functional performance (Table 4.8). There were no significant relations between subject age and functional capacity.

Table 4.7. Significant correlations between objective functional capacity and subject height

Comparison		p value	r value
25m-Walk Test			
	Baseline	0.001	-0.716
	Week 8	0.001	-0.700
Stair-Climb Test			
	Baseline	0.000	-0.852
	Week 8	0.000	-0.867
Chair-Rise Test			
	Baseline	0.010	-0.574
	Week 8	0.006	-0.606

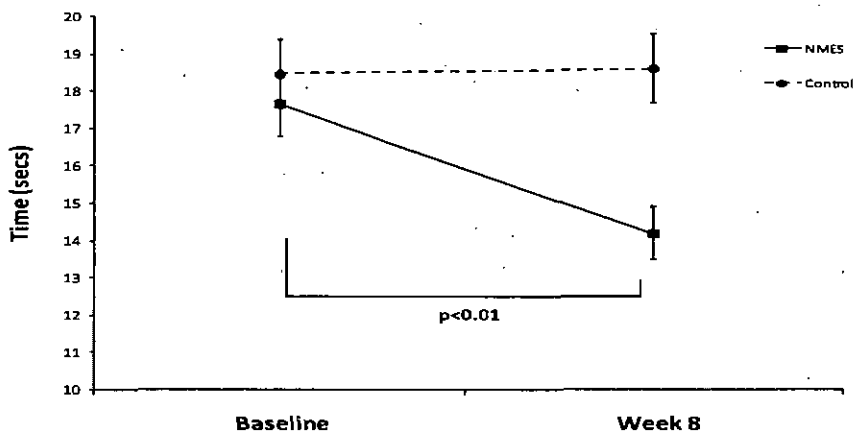
p value – significance level; r value – correlation coefficient

Table 4.8. Significant correlations between objective functional capacity and subject BMI

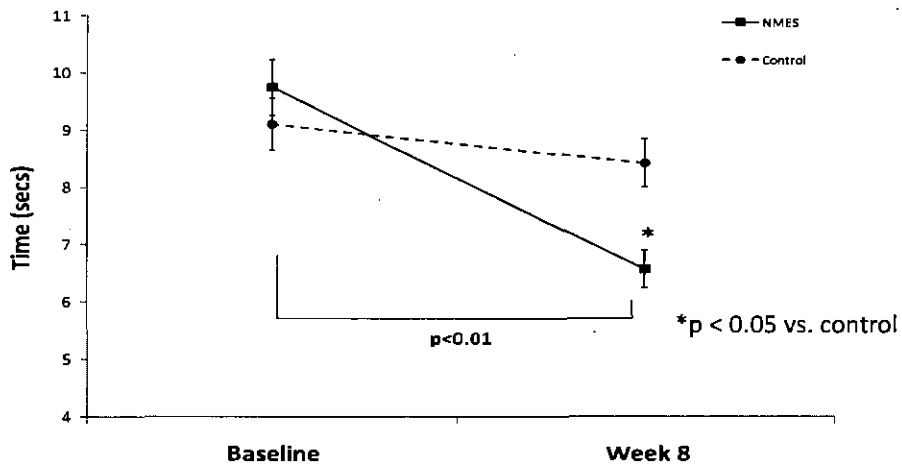
Comparison		p value	r value
25m-Walk Test			
	Baseline	0.004	0.632
	Week 8	0.046	0.435
Stair-Climb Test			
	Baseline	0.009	0.582
	Week 8	0.018	0.526
Chair-Rise Test			
	Week 8	0.040	0.450

p value – significance level; r value – correlation coefficient

a) Timed stair-climb test (TST)



b) Timed chair-rise test (TCT)



c) Timed 25-metre walk test (TWT)

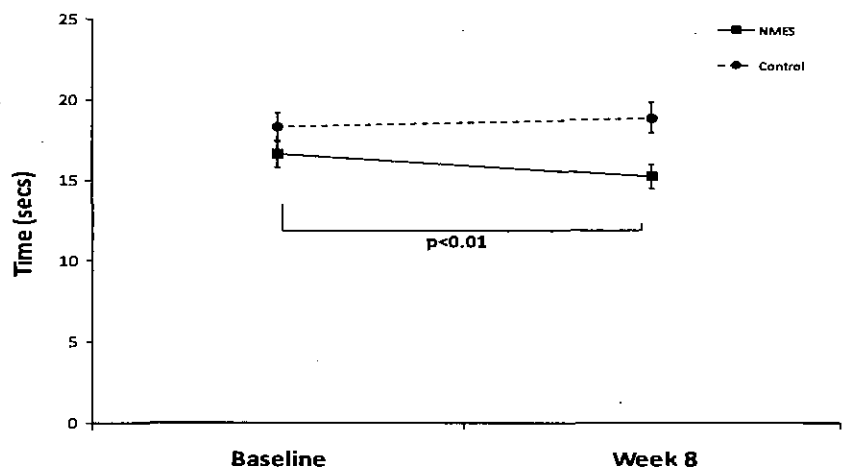


Figure 4.2. Objective function

4.3.5 Anthropometric Evaluation

Thigh circumference was similar in both groups and did not change from baseline to week 8 (Table 4.9). The Con group had greater (p=0.038) knee ROM than the NMES group at baseline. At wk 8 there was no statistical difference (p=0.08). Extension lag at baseline was greater (p=0.012) in the NMES group compared to the Con group. Despite decreasing significantly over the study period in the NMES group (33%, p=0.043), it was still greater (p=0.024) than the Con group. Thigh girth was directly related to subject age, height, and BMI (Table 4.10). Knee ROM had an inverse relation with BMI at baseline (r^2=0.29; p=0.016).

Table 4.9. Clinical evaluation

	Time	
	Baseline	**Week 8**
Extension Lag (degrees)		
NMES	10.3 ± 8.1 *	6.9 ± 5.6 [a]*
Control	0.0 ± 0.0	0.3 ± 0.8
Range of Motion (degrees)		
NMES	106.0 ± 16.8 *	107.8 ± 17.6
Control	122.0 ± 12.1	121.7 ± 13.1
Thigh Circumference (cm)		
NMES	47.8 ± 3.4	47.9 ± 2.8
Control	50.2 ± 5.6	50.6 ± 5.9

Values are means ± SD ; [a] <0.05 vs. Baseline; *p < 0.05 vs. control

Table 4.10. Significant correlations between thigh girth and subject demographics

Comparison		p value	r value
Age			
	Baseline	0.014	-0.547
	Week 8	0.010	-0.573
Height			
	Baseline	0.033	-0.470
	Week 8	0.042	-0.445
Body Mass Index (BMI)			
	Baseline	0.000	0.796
	Week 8	0.000	0.897

p value – significance level; r value – correlation coefficient

4.3.6 Self-Report Outcome Measures

The WOMAC and Oxford knee scores of the Con group and all outcome scores of the NMES group did not change significantly over the study period. SF-36 component scores of the Con group improved from baseline to wk 8 (Figure 4.3): physical health (18.8%, p=0.028) and mental health (16.2%, p=0.046). There was no difference between experimental groups in any outcome measure at baseline and wk 8.

There were no significant associations between subject height and any outcome measure. The WOMAC pain subscale related to subject age at baseline only (r^2=0.25; p=0.024). BMI had a direct relation with WOMAC stiffness at wk 8 (r^2=0.21; p=0.037), and an inverse relation with SF-36 physical health scores at baseline (r^2=0.20; p=0.04) and wk 8 (r^2=0.20; p=0.04).

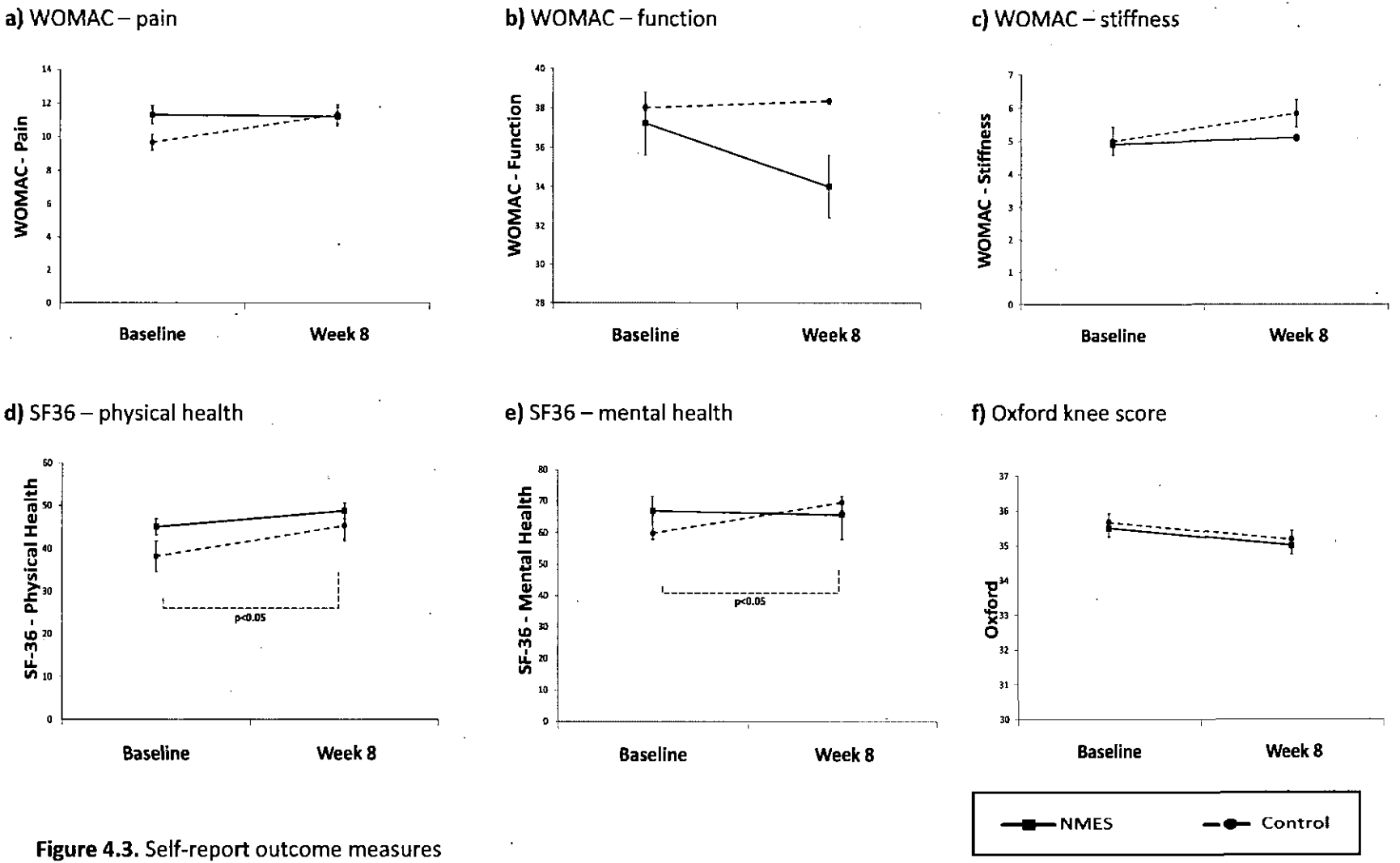


Figure 4.3. Self-report outcome measures

4.3.7 *Significant* Correlations Between Outcome Measures

Performance of the TST was strongly related to QFM strength in the involved limb (Table 4.11). Involved QFM strength only related to TWT at baseline and did not correlate with the TCT. HS peak torque of the involved limb related to performance of the TST and TWT. Greater QFM and HS strength in the uninvolved limb were strongly associated with better functional capacity (Table 4.12).

Muscle strength did not relate to subjective outcomes determined by WOMAC, SF-36 mental health scores and Oxford knee scores. SF-36 physical health scores related to involved limb *isokinetic HS strength* (60°/sec) at baseline (r^2=0.19; p=0.047) and wk 8 (r^2=0.29; p=0.016). There were no significant correlations between the WOMAC subscales and objective functional capacity. SF-36 physical health scores were inversely related to functional capacity (Table 4.13). SF-36 mental health and Oxford knee scores related to the TWT at baseline.

QFM strength did not relate to extension lag or thigh circumference and there were only moderate associations found between QFM strength and knee ROM (Table 4.14). Self-report outcome scores did not relate to knee ROM or extension lag. Thigh circumference related to WOMAC stiffness at baseline (r^2=0.24; p=0.026) and wk 8 (r^2=0.23; p=0.029). Only thigh circumference correlated with objective functional capacity (Table 4.15)

25-Metre Timed Walk Test

A pre-measured 35 metre well lit, tilled, low traffic, indoor hallway with the distances clearly marked was used for the 25-metre timed walk test (TWT). This provided a 5 metre extension at either end to allow for acceleration and deceleration of gait, aiming to achieve a constant velocity during the 25 metre distance. Only the time taken to walk the central 25 metre distance was recorded. Subjects were instructed to "on the word 'Go' walk 35 metres to the marked line as quickly and as safely as you can". They were allowed to stop or use a mobility aid if required.

Figure 3.10. Testing hallway for 25-metre timed walk test

3.9.2 Timed Stair-Climb Test

A well-lit, low traffic indoor stairwell was used for the timed stair-climb test (TST). The single flight of stairs involved 11 steps, each with an 18 cm rise and 30 cm depth. Subjects were asked to use no handrails during the test if possible although 1 was available. They were instructed to "climb up, turn and descend 11 steps as safely and as quickly as you can". Test-retest reliability has been previously found to be 0.88.(261)

Table 4.11. Significant correlations between involved muscle strength and objective function

Comparison		p value	r value
Timed Walk Test (TWT)			
Quadriceps Isokinetic 60°/sec			
	Baseline	0.013	-0.553
Quadriceps Isokinetic 120°/sec			
	Baseline	0.004	-0.635
Quadriceps MVIC			
	Baseline	0.002	-0.665
Hamstring Isokinetic 60°/sec			
	Baseline	0.002	-0.676
	Week 8	0.043	-0.442
Hamstring Isokinetic 120°/sec			
	Baseline	0.001	-0.738
	Week 8	0.014	-0.546
Timed Stair-Climb Test (TST)			
Quadriceps Isokinetic 60°/sec			
	Baseline	0.008	-0.591
	Week 8	0.028	-0.488
Quadriceps Isokinetic 120°/sec			
	Baseline	0.002	-0.679
	Week 8	0.006	-0.614
Quadriceps MVIC			
	Baseline	0.004	-0.644
	Week 8	0.007	-0.603
Hamstring Isokinetic 60°/sec			
	Baseline	0.008	-0.591
	Week 8	0.004	-0.636
Hamstring Isokinetic 120°/sec			
	Baseline	0.003	-0.653
	Week 8	0.001	-0.699
Timed Chair-rise Test (TCT)			
Hamstring Isokinetic 120°/sec			
	Baseline	0.018	-0.525
	Week 8	0.032	-0.474

p value – significance level; r value – correlation coefficient

Table 4.12. Significant correlations between uninvolved muscle strength and objective function

Comparison		p value	r value
Timed Walk Test (TWT)			
Quadriceps Isokinetic 60°/sec			
	Baseline	0.000	-0.806
	Week 8	0.000	-0.797
Quadriceps Isokinetic 120°/sec			
	Baseline	0.000	-0.756
	Week 8	0.000	-0.747
Quadriceps MVIC			
	Baseline	0.000	-0.838
	Week 8	0.000	-0.759
Hamstring Isokinetic 60°/sec			
	Baseline	0.000	-0.821
	Week 8	0.001	-0.692
Hamstring Isokinetic 120°/sec			
	Baseline	0.000	-0.765
	Week 8	0.000	-0.776
Timed Stair-Climb Test (TST)			
Quadriceps Isokinetic 60°/sec			
	Baseline	0.000	-0.838
	Week 8	0.000	-0.903
Quadriceps Isokinetic 120°/sec			
	Baseline	0.000	-0.865
	Week 8	0.000	-0.868
Quadriceps MVIC			
	Baseline	0.000	-0.909
	Week 8	0.000	-0.879
Hamstring Isokinetic 60°/sec			
	Baseline	0.000	-0.894
	Week 8	0.000	-0.770
Hamstring Isokinetic 120°/sec			
	Baseline	0.000	-0.885
	Week 8	0.000	-0.833
Timed Chair-rise Test (TCT)			
Quadriceps Isokinetic 60°/sec			
	Baseline	0.001	-0.702
	Week 8	0.002	-0.676
Quadriceps Isokinetic 120°/sec			
	Baseline	0.001	-0.696
	Week 8	0.008	-0.591
Quadriceps MVIC			
	Baseline	0.001	-0.702
	Week 8	0.001	-0.712
Hamstring Isokinetic 60°/sec			
	Baseline	0.000	-0.765
	Week 8	0.052	-0.422
Hamstring Isokinetic 120°/sec			
	Baseline	0.004	-0.634
	Week 8	0.013	-0.553

p value – significance level; r value – correlation coefficient

Table 4.13. Significant correlations between subjective outcome scores and objective function

Comparison		p value	r value
SF-36 Physical Health Component Score			
Timed Walk Test (TWT)			
	Baseline	0.003	-0.659
	Week 8	0.011	-0.564
Timed Stair-Climb Test (TST)			
	Week 8	0.015	-0.545
Timed Chair-Rise Test (TCT)			
	Baseline	0.031	-0.476
SF-36 Mental Health Component Score			
Timed Walk Test (TWT)			
	Baseline	0.013	-0.552
Oxford Knee Score			
Timed Walk Test (TWT)			
	Baseline	0.032	0.473
Timed Chair-Rise Test (TCT)			
	Baseline	0.029	0.482

p value – significance level; r value – correlation coefficient

Table 4.14. Significant correlations between involved muscle strength and knee range of motion

Comparison		p value	r value
Quadriceps Isokinetic 60°/sec			
	Baseline	0.021	0.531
	Week 8	0.034	0.467
Quadriceps Isokinetic 120°/sec			
	Baseline	0.045	0.438
	Week 8	0.017	0.529
Quadriceps MVIC			
	Baseline	0.045	0.438
Hamstring Isokinetic 60°/sec			
	Baseline	0.024	0.500

p value – significance level; r value – correlation coefficient

Table 4.15. Significant correlations between thigh girth and objective function

Comparison		p value	r value
Timed Walk Test (TWT)			
	Baseline	0.017	0.533
Timed Stair-Climb Test (TST)			
	Baseline	0.017	0.534
	Week 8	0.042	0.444
Timed Chair-Rise Test (TCT)			
	Week 8	0.048	0.429

p value – significance level; r value – correlation coefficient

3.4 DISCUSSION

There is little published data regarding NMES as a training modality in knee OA. This is the first study that has specifically evaluated its use in patients with end stage disease. We found that 8 weeks unilateral NMES training increased quadriceps and hamstring strength in both limbs of subjects with advanced knee OA with associated improvements in objective functional capacity. However, it produced only limited changes in subjective outcome scores. Patients who underwent NMES training developed greater tolerance for higher stimulation intensities over the study period.

Compliance with NMES training was excellent. Stimulator recorded compliance was greater than 100% in several subjects indicating they had used the device more often than advised. This was mainly seen during the two week conditioning period and may simply represent subjects familiarising themselves with the stimulator. During the final three weeks, there was inconsistency between logbook reported (99%) and stimulator recorded (92%) compliance. Clearly some subjects were over-reporting their usage. This was most evident in two male subjects who documented compliance rates at more than 95% in their logbooks whereas actual usage was less than 71%. Reasons reported by subjects for non-adherence include travelling at a time when they were due to have a stimulation session or forgetting to bring the charger for the stimulator and also simply forgetting to perform a training session.

Talbot et al reported a lower compliance level of 81% when using a 12 week unsupervised home-based NMES program in subjects with moderate knee OA.(247) Compliance tends to decline over the course of a training program and their longer study duration would explain, in part, the lower rate. The NMES device we used was garment-based rather than consisting of separate electrodes, wires, and stimulation packs making it more amenable for training. In addition our subjects were participating in a preoperative training program aiming to facilitate postoperative recovery after TKA. As such they may have been more motivated to adhere to the NMES program.

Incorporating a conditioning period at the start of NMES training has been recommended to allow subjects develop tolerance for higher stimulation intensities.(218) This had a significant effect in our study where the NMES group subsequently tolerated more stimulus than the control group. There was further improvement in tolerance from week 2 to week 5 in the NMES group seen as an increase in the stimulator recorded training intensity.

It has been generally accepted that for NMES to increase quadriceps muscle strength, subjects must achieve an induced contraction threshold (MTIC) equivalent to 25-50% of their volitional peak torque (MVIC).(218;247;256;276) Even though there was much individual variability, the MTIC of both groups was generally greater than 25% MVIC throughout the study. At baseline, seven subjects in the NMES group had an MTIC >25% MVIC. Following the conditioning period, the %MVIC of six subjects improved. However, it deteriorated in four subjects despite increases in electrically induced contraction force. This was due to them experiencing greater increases in volitional strength (i.e. increases in MVIC > increases in MTIC). By week 8, seven subjects of the NMES group had a MTIC >25% MVIC. Of the three subjects in the NMES group that were below the 25% threshold level, one elderly female subject never produced more than 18% MVIC despite tolerating maximal stimulation intensity. The other two male subjects tolerated maximal stimulation intensity at all time points and also experienced the greatest increases in quadriceps strength.

Given that strength increased despite subjects not achieving the 25% MVIC threshold, we consider a lower level of stimulus to be clinically effective in weakened subjects with end stage knee OA. Using regression analysis, Talbot et al determined a level of 18% MVIC to be sufficient for quadriceps strength gain in a population with knee OA.(247)

Subjects with a higher BMI had lower stimulation tolerance in their involved limb. Pain felt in response to NMES has been attributed to direct stimulation of afferent nerve endings (C fibres) as well discomfort from the resulting muscle contraction itself.(277) Subjects with increased BMI may have a greater proportion of these free nerve endings which would limit

stimulation tolerance.(218) Although we did not have sufficient numbers to analyse for gender differences, females typically have lower sensory thresholds than men, reporting greater pain at low stimulation intensities.(219)

Significant differences in quadriceps strength existed at baseline between limbs with the involved side up to 32% weaker. This concurs with previous studies performed on subjects undergoing arthroplasty for end-stage hip and knee OA where differences of 20-22% have been reported.(154;155;260;278) There was no significant increase in muscle strength seen in the control group. In contrast the NMES group increased involved limb QFM isokinetic peak torque at 60°/sec by 33% and isometric strength by 26% so that by the end of the study there was no significant difference in QFM strength between limbs.

Talbot et al performed a 12-week home-based NMES program in subjects with knee OA, increasing isometric QFM strength by 9%.(247) They utilised NMES 3 days·wk^{-1} with stimulation intensity progressively increased to predetermined levels every 4 weeks. In our study, subjects increased stimulation intensity to their maximal tolerated level during the initial two weeks and subsequently trained for 6 weeks (5 days·wk^{-1}) at their highest tolerated intensities. The greater training stimulus in our study may explain the superior strength gains we observed despite it having a shorter duration. Durmus et al compared NMES and exercise training over a 4 week period (5 days·wk^{-1}) in subjects with knee OA although excluded individuals with end-stage disease.(248) Both modalities produced similar QFM strength gains of approximately 35%.

Involved limb hamstring peak torque also increased in the NMES group to an extent that there was no difference in strength when compared with the uninvolved limb by the end of the program. NMES may have altered neural signalling in spinal motor efferents to the hamstring muscle group, increasing its force generating ability. The increases in hamstring strength were also greater than gains in quadriceps strength. Such a pattern of strength gain has been reported in subjects with knee OA following 3 months of exercise rehabilitation

where hamstring strength increased by 29% compared to 14% for the quadriceps.(188) These findings contrast with studies of exercise training on healthy subjects where quadriceps strength increases to a greater extent than hamstring strength.(92)

Although to a lesser degree, muscle strength also increased in the uninvolved limb of the NMES group suggesting a "crossover effect".(202) Studies have reported that quadriceps weakness and activation failure affect limbs bilaterally in a variety of knee conditions, suggesting inhibition from central neurological pathways in response to gamma-loop dysfunction on the affected side.(156;164;279;280) Normal gamma loop function is required for a subject to produce maximal volitional muscle activation. Thus when afferent signals from the joint (Ia neurons) are altered, high threshold motor units (type II fibres) are not recruited resulting in muscle activation deficits.(281;282) Konishi et al suggested that the contralateral leg may be affected due to inhibitory signals from afferents of the involved side reducing contralateral signalling via interneurons in the spinal cord.(282) Contralateral strength gain may be an indirect result of NMES training where it reduces inhibitory signals from both central spinal efferents and peripheral afferents. This is purely theoretical and warrants further investigation. It could also be argued that the increase in strength on the affected side may simply have afforded greater activity, permitting actual strength gain through an increase in overall physical function.

Fisher et al performed several studies of exercise training on subjects with knee OA reporting quadriceps strength gains of 8–55%.(150;186-188;191) Many trials found that the greatest improvements in muscle strength occurred within the first 4 weeks of training. In this study we saw the greatest increases in strength within the first two weeks of training (after the conditioning period). These initial gains may be partly attributable to motor learning of the dynametric testing procedure. Such a phenomenon would explain why the control group, although not significant, had greater muscle strength at week 2 compared to baseline.

However, only the NMES group increased muscle strength beyond week 2 confirming the training effect of NMES.

Although healthy obese people have greater absolute quadriceps strength than lean adults, possibly due to their increased weight acting as a training stimulus, this is reversed when adjusted for body mass resulting in a relative strength deficit (175) Subjects with knee OA are generally more obese than healthy adults and the deficit in strength is even greater.(145;149) This study confirms that BMI is inversely related to quadriceps muscle strength in subjects with knee OA although it does not appear to relate to hamstring strength. Surprisingly, subject age did not relate to muscle strength in this study, clearly demonstrating the detrimental effect that end-stage knee OA has on muscle strength in middle-aged and elderly people.

Improvements in functional capacity were only seen in the NMES group. Walking time improved by 9% while greater gains of 20% and 33% were seen for the timed stair-climb and chair-rise tests respectively. Substantial improvement in the timed chair-rise test was such that the NMES group was 22% better compared to the control group at the end of study. Taller subjects performed better in the functional assessments unlike individuals with greater BMI's. Female gender also predisposes to poorer functional performance.(153) In subjects with knee OA, Durmus et al reported decreases in stair-climb and 50-metre walk times of 19% and 14% respectively following 4 weeks of NMES training.(248) Conversely, Talbot et al did not find any improvement in stair-climb or 100-foot walk-turn-walk tests following 12 weeks NMES training in adults with knee OA.(247) However, like us, they saw significant improvement in chair-rise times. Adherence to a training program must be good if subjects with knee osteoarthritis are to improve their physical function, and the superior results seen in this study compared to Talbot et al may simply be due to better compliance.(193) Schilke et al applied an exercise training program to a cohort with knee OA, also finding that changes in walking time were not as substantial compared to other functional measures.(283)

Quadriceps strength usually correlates with functional performance in healthy elderly adults.(105) We found strong correlations between all measures of functional capacity and QFM strength in the uninvolved limb. However there were only strong associations found between involved QFM strength and stair-climbing times throughout the study period. Involved QFM strength did not correlate with chair-rise times. This contrasts with Taffe et al who found that changes in muscle strength following 6 months exercise training in healthy subjects strongly correlated with chair rise times.(139) While it is unclear as to why healthy and arthritic adults produce differing associations, it is not unusual. Gur et al described only small to moderate correlations between strength and function in a cohort with bilateral knee OA.(284) Instead they found that differences in the ratio of hamstring to quadriceps torque explained better the variations in objective function, primarily stair climbing.(284)

Although the greatest improvements were seen for the chair-rise test, and as such it may be the most sensitive measure of functional performance in subjects with knee OA, we recommend that the stair-climb test be performed also in future studies given the strength of its association with quadriceps muscle strength.

An extension lag was only present in the NMES group at baseline. Since both groups had comparable muscle weakness at baseline it is unclear as to why the control group did not demonstrate a similar deficit. Nonetheless, the degree of extension lag reduced significantly in response to NMES training. There was also evidence that NMES may facilitate an increased knee range of motion. The NMES group had a lower ROM at baseline compared to the control group but at the end of the program there was no statistical difference between groups.

Although not statistically significant, the NMES group reported a 16% improvement in WOMAC function scores. The WOMAC pain and stiffness scores did not change in either group. Durmus et al found improvements of more than 60% in the three WOMAC subscales in subjects with early to moderate knee OA after NMES training.(248) While our study produced comparable improvement in objective measures of functional capacity, there is clearly a

discrepancy regarding subjective outcome scores. Our cohort consisted of subjects with end-stage disease whereas Durmus et al excluded such candidates. Self-report measures strongly relate to knee pain whereas objective performance measures relate to self-efficacy.(262) Subjects with end-stage knee osteoarthritis have greater knee pain than those with less severe disease and although we found gains in strength and objective function, patient perceived pain did not change. This would explain the limited changes in WOMAC function and stiffness scores of both experimental groups in our study. These findings compare favourably with the study of NMES in knee OA from Talbot et al who also did not find any improvement pain despite gains in QFM strength and objective function.(247)

Only the control group had a significant improvement in both SF-36 component scores. This is surprising, especially since the NMES group had substantial strength and functional improvement. This study may have been underpowered to detect significant change in the NMES group, thereby representing a type 2 error. The Oxford knee score did not change in either group. This outcome instrument was originally designed to reflect symptoms in patients undergoing TKA. It can be influenced by hip and spinal pathology thereby limiting its specificity.(268;285) It may also lack the sensitivity required to determine change in response to NMES or exercise training and we would not recommend its use in further studies not involving knee surgery.

Quadriceps muscle strength did not relate to any subjective outcome measure and there were only a few significant correlations between objective functional capacity and the self-report outcome scores. Most notably, no associations were found with any WOMAC score. Maly et al found only moderate relations between self-report scores (WOMAC and SF-36) and functional performance measures in subjects with knee OA, suggesting that different areas of disability are examined by each.(262)

The strengths of this study include its design as a randomised control trial and that all assessments were performed with the respective assessor blinded to subject group

assignment. A limitation of isokinetic muscle strength determination is that the external lever arm of the dynamometer is fixed whereas the centre of rotation of the knee changes during its arc of motion. As such, the recorded strength can over or underestimates the true force generated.(12) Furthermore the testing order was not randomised with isokinetic assessment followed by isometric and then MTIC at each evaluation point. This order was chosen because isometric is more fatiguing than isokinetic testing and stimulated contractions may provoke greater muscle damage and soreness than voluntary exercise.(286)

Patients were not discouraged from performing additional exercises preoperatively in either group. Given this was an unsupervised program, it could be suggested that some subjects in the NMES group combined their NMES training with voluntary exercise, thereby confounding the true effect of muscle stimulation.

A limitation of performing physical assessments with small sample sizes and in older subjects is the applicability of pertinent findings to the greater population. Despite the small sample size, we found significant between group training effects on functional capacity. There may have been insufficient statistical power to realise the full benefits of NMES, specifically in terms of subjective outcomes. This work can be considered a feasibility and short-term efficacy study. We advise further research on a broader patient cohort with the inclusion of cost-effectiveness study before the widespread implementation of NMES in subjects with knee OA can be recommended.

We have shown that an 8 week unsupervised home-based NMES program can safely and effectively improve performance in functional tasks in subjects with end-stage knee OA. This was associated with significant gains in quadriceps and hamstring muscle strength in the involved limb. There was also a cross-over effect as strength also increased in the untrained limb. Stimulation tolerance improved during the course of the program with most subjects being able to train at the maximum stimulator output. While healthy subjects require electrically induced contraction force of at least 25% to increase muscle strength, we have

seen that lower values can produce significant gains in weakened subjects with advanced knee OA. The potential role of this modality in subjects with knee OA should be further evaluated as it offers a method of improving function in this population. NMES may offer definitive therapy in subjects with end-stage knee osteoarthritis in whom TKA is declined or deemed unsuitable.

Chapter V

Quadriceps Femoris Neuromuscular Electrical Stimulation in Knee Osteoarthritis: The Mechanisms Associated with Strength Gain

5.1 INTRODUCTION

Transcutaneous neuromuscular electrical stimulation (NMES) has been employed as both a rehabilitation tool in injured individuals as well as a training modality in athletes either alone or as an adjunct to volitional exercise.(234;241) A systematic review of randomised controlled trials concluded that NMES can increase strength in healthy and impaired muscle compared with no exercise.(234) Compared with volitional exercise, NMES was considered superior at preserving muscle strength and mass during cast-immobilisation and may be preferential in cases where compliance with voluntary exercise is poor.(234)

The exact mechanisms underlying strength improvements with NMES have not been fully elucidated, and limited data is available regarding changes in muscle at the molecular level. Early evidence suggested a reversed order of fibre recruitment than that seen during voluntary contractions such that larger fast-twitch (type II) fibres were preferentially recruited first followed by smaller slow-twitch (type I) fibres.(204) Recent work indicates that although NMES may favour the recruitment of fast-twitch fibres, it is in fact due to random activation of motor units.(202;211)

The principle mechanism behind quadriceps femoris muscle weakness in aging adults is a decrease in muscle mass (sarcopenia).(26;96) Healthy individuals often have no significant difference in strength between limbs, although asymmetric lower limb weakness can adversely affect balance increasing the risk of falls.(26;96;102;287) Muscle specific force, calculated as force per unit muscle cross-sectional area (force/CSA), should remain constant for strength decline to be explained by muscle atrophy alone.(12;13;15) In healthy subjects

aged over 65 years, strength loss is greater than muscle atrophy implicating the adverse effects of neuromuscular adaptation.(12)

In addition to sarcopenia, disuse atrophy and neuromuscular activation failure, unilateral strength deficits of up to 44% have been reported in subjects with knee osteoarthritis (OA).(149;152-155;288) Petterson et al reported that muscle activation failure contributes more than muscle atrophy in quadriceps weakness associated with end-stage knee OA and suggested that activation deficits arise first and potentiate later atrophy.(154) The authors also found a 7% decrease in specific force of the involved limb.(154) Conversely, Lewek et al reported atrophy to be the principle cause of quadriceps weakness in knee OA.(166) This discrepancy may depend on the varying disease severity in the cohorts studied. Pap et al reported that while subjects with advanced disease had less activation failure than those with moderate disease their overall force generating capacity was lower.(152) In addition, higher levels of activation failure are associated with poorer physical function.(163)

Progressive resistance training produces changes in muscle strength that are greater than increases in CSA in subjects with no knee pathology as.(112;117) This results in an increase in specific force and infers that healthy individuals also suffer activation deficits although to a lesser degree than subjects with knee OA, which are decreased with exercise. Improvements in quadriceps neuromuscular activation by up to 20% have also been found in subjects with knee OA and post-total knee arthroplasty following exercise training.(190;289) Though studies have reported strength gains following NMES in knee osteoarthritis, to our knowledge none have described changes in muscle CSA or specific force.(247;248)

Myosin heavy chain (MHC) is the principle contractile protein by which muscle fibre types is classified: MHC I (slow), MHC IIa (fast), and MHC IIx (fast). Disuse and immobilisation are associated with a slow to fast transformation in MHC gene and protein expression.(60;69;290) Following endurance training, there is a shift from fast to slow MHC isoform expression. A six-week programme resulted in decreased levels of MHC IIx mRNA

whereas a 16 week program found MHC IIx mRNA expression decreased by 50%, with a concomitant increase in MHC I and IIa mRNA levels of 63% and 99% respectively.(18;128) To our knowledge, no studies have examined changes in MHC mRNA isoform expression in response to NMES training. Given that NMES stimulates type II fibres more than exercise training alone, could NMES increase MHC IIa and IIx mRNA expression? A shift from a fast to slow MHC genotype was evidenced in the tibialis anterior muscle of six subjects with spinal cord injury following chronic (6 to 12 months) low intensity stimulation.(291) Changes in MHC gene expression in healthy or osteoarthritic subjects following NMES training are not well understood.

Muscle mass homeostasis is maintained by a fine balance between protein synthesis and degradation with the regulatory molecular mechanisms only beginning to be understood. Central to hypertrophy is Insulin-like growth factor 1 (IGF-1) which acts via the P13K/AKT pathway to induce protein synthesis and inhibit protein breakdown.(70) While several pathways have been implicated in disuse atrophy, the bulk of myofibrillar proteolysis (actin and myosin) involves the ubiquitin-proteasome system (UPS) with many steps upregulated in muscle wasting diseases.(76) Within the UPS two muscle-specific E3 ubiquitin ligases seem to play an important role although their association with MHC expression is unclear: muscle-specific F-box (MAFbx) and muscle specific RING finger 1 (MURF-1).

While there is growing understanding for alterations in neuromuscular adaptations following NMES, much has yet to be elucidated. We hypothesised that NMES would increase quadriceps femoris muscle (QFM) strength more than CSA, producing an increased specific force in a cohort with end-stage knee OA. In addition we expected a slow to fast shift in MHC genotype, an increase in IGF-1 mRNA and a decrease in MURF-1 and MAFbx mRNA.

5.2 PATIENTS AND METHODS

5.2.1 Patients

This study conformed to the Helsinki declaration with full approval given by the local ethics review committee. Between July and October 2007, men and women aged between 45 and 80 years undergoing TKA for advanced primary knee OA were recruited from a preoperative orthopaedic assessment clinic. Allocation to either intervention (NMES) or control (Con) groups was performed using block randomisation and sealed numbered envelopes. Informed consent was obtained from patients after they received individual counselling on the full nature of the study. Seventeen patients enrolled in the study. One control subject withdrew due to marked deterioration in her preoperative clinical condition. A subject from the intervention group did not have imaging performed at week 8, and was excluded from this analysis. There was no difference between groups in any parameter at baseline (Table 5.1).

Table 5.1. Baseline characteristics

	Group		
	NMES	**Control**	**p value**
Male: Female	3 : 6	2: 4	
Age (y)	65.6 ± 7.4	64.8 ± 11.0	0.891
Height (cm)	159.5 ± 9.7	158.0 ± 7.2	0.764
Weight (kg)	78.8 ± 11.4	79.1 ± 13.1	0.964
BMI (kg/m^2)	31.0 ± 2.9	31.8 ± 6.1	0.753

Values are means ± SD; BMI: Body Mass Index

5.2.2 Overview

Subjects in the intervention group received 8 weeks of NMES training applied unilaterally to the QFM of the affected side as an unsupervised, home-based program. Subjects assigned to the active control group received 8 weeks standard preoperative care simply consisting of advice on knee range of motion and muscle strengthening exercises. QFM strength and CSA were evaluated at baseline and wk 8. Expression of genes associated with muscle hypertrophy (IGF-1) and atrophy (MAFbx & MURF-1) as well as expression of MHC isoform mRNA, were determined at baseline and wk 8.

5.2.3 Intervention – Neuromuscular Electrical Stimulation

A portable stimulator (KneeHAB II, Bio-Medical Research, Galway, Ireland) was used for 20 min·day^{-1} on alternate days for two weeks followed by 5 days·wk^{-1} for 6 weeks as a quadriceps strengthening program. Patients were encouraged to train at their highest tolerated intensity for as long as possible during each session.

5.2.4 Evaluation Protocols

5.2.4.1 Quadriceps Muscle Strength

QFM peak torque of both involved and uninvolved limbs were determined using a clinical dynamometer (Biodex Medical Instruments, Shirley, NY). Concentric isokinetic QFM strength was evaluated at angular velocities of 60°/sec and 120°/sec. Isometric QFM strength (MVIC) was assessed with the knee at 60 degrees of flexion. Standardised verbal coaching was given by a research assistant blinded to each participant's group assignment with the maximum force generated in each test recorded as the peak torque.

5.2.4.2 Quadriceps Muscle Cross-Sectional Area (CSA)

A Gyroscan Intera 1.5T MRI scanner (Philips Medical Systems, Holland) was used to radiologically determine QFM cross-sectional area (CSA) of both involved and uninvolved

limbs. A coronal scan identified the greater trochanter and lateral knee joint line with axial imaging performed at their mid-point. Manual planimetry was then used to calculate QFM CSA (cm^2).

5.2.4.3 Quadriceps Muscle Specific Force (F^0)

QFM specific force (Nm/cm^2) of the involved and uninvolved limbs was calculated from QFM peak torque and CSA measurements using the following equation:(12;13)

$$F^0 (Nm/ cm^2) = \text{QFM Peak Torque (Nm)} / \text{CSA } (cm^2)$$

Specific force was determined from the corresponding isokinetic strength assessments and recorded as F^0 (60°/sec) and F^0 (120°/sec) when. The MVIC specific force was recorded as F^0 (0°/sec)

5.2.4.4 Gene Expression

Approximately 100 mg of muscle was obtained from *m. vastus lateralis* of the affected limb at baseline and week 8 using the suction needle biopsy technique. These were flash frozen in liquid nitrogen and stored at -80°C. Samples of 25-30 mg muscle were homogenised and mRNA extraction performed using the TRI reagent method. One microgram of mRNA from each sample was reverse transcribed to cDNA using the reverse transcription system (A3500; Promega, Madison, WI) and stored at -20° until further analysis.

RT-PCR was performed using an ABI Prism 7500 Sequence Detection System (Applied Biosystems, Foster City, CA). Pre-designed gene-specific primer and probes for MHC-1 (MYH7), MHC-IIa (MYH2), and MHC IIx (MYH1) as previously described, and GAPDH was used as the endogenous control to determine relative expression of the target mRNA (cDNA). Each sample was run in duplicate with the following steps: one cycle at 50°C for 2 min; a denaturing cycle at 9S°C for 10 min; 40 cycles of denaturing at 95°C for 15 sec; and annealing and elongation at 60°C for 1 min. The mRNA (cDNA) content of both the target genes and GAPDH was calculated from a corresponding standard curve constructed using serial dilutions of mRNA pooled from

all samples. The mRNA data was then expressed as the ratio between the gene of interest and GAPDH.

5.2.5 Statistical Analysis

Independent t-tests were used to evaluate group differences at baseline. The Wilcoxan signed ranks tests compared within group differences and the Mann Whitney test performed between group comparisons. The relation between selected parameters was determined using Spearman rho correlations. Statistical significance was set at p=0.05. Statistical tests were performed using the Statistical Package for Social Sciences version 15.0 (SPSS, Inc., Chicago, IL).

5.3 RESULTS

5.3.1 Muscle Strength

There was no difference in QFM peak torque between groups at any time point. Isometric and isokinetic peak torque at 60°/sec was higher at wk 8 compared to baseline in both limbs of both groups (Figure 5.1). Isokinetic strength increased in the Con group at 120°/sec. There was no baseline difference in QFM strength between limbs in the Con group. QFM strength of the uninvolved limb was greater than the uninvolved limb in the NMES group at baseline; isokinetic 60°/sec, 35.4% (p=0.015); isokinetic 120°/sec, 26.6% (p=0.066); MVIC, 22.3%, (p=0.066). There was no difference in strength between limbs of either group at wk 8. Muscle strength was related to subject height but not age or BMI (Table 5.2).

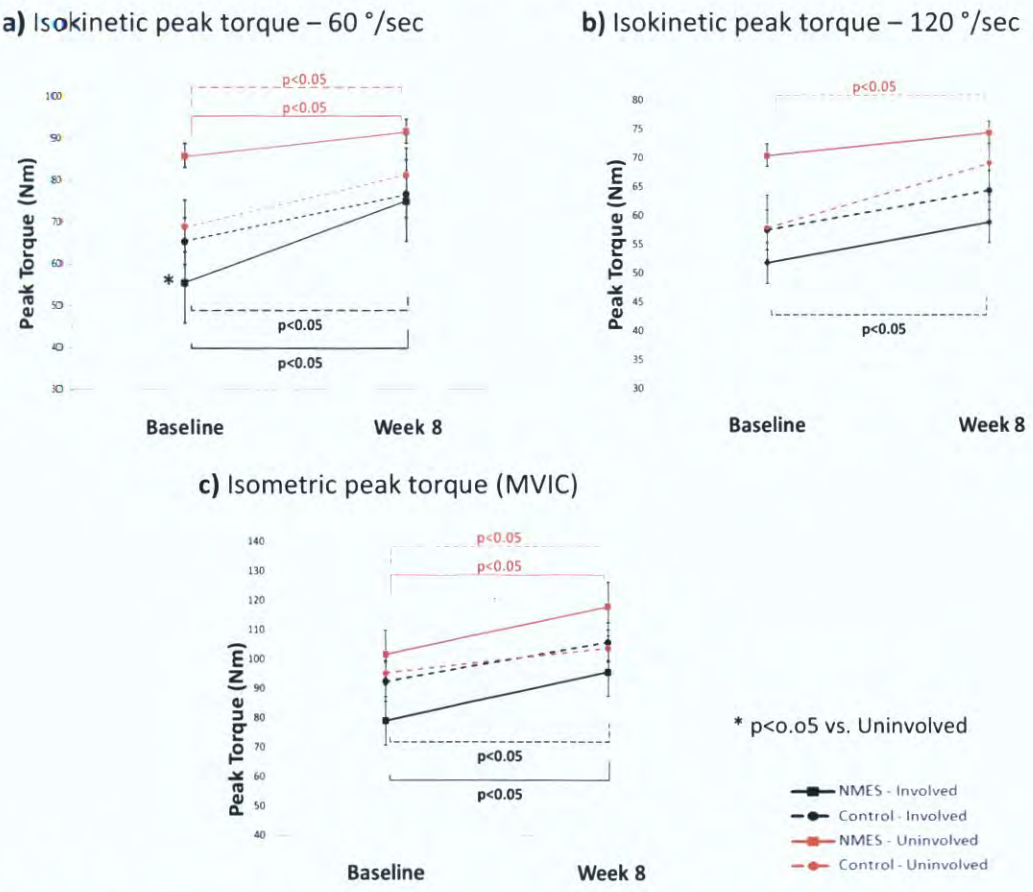


Figure 5.1. Quadriceps muscle strength

Table 5.2. Significant correlations between muscle strength and subject height

Comparison		p value	r value
INVOLVED LIMB			
Quadriceps Isokinetic 60°/sec			
	Baseline	0.017	0.548
	Week 8	0.039	0.469
Quadriceps Isokinetic 120°/sec			
	Baseline	0.002	0.700
	Week 8	0.012	0.580
Quadriceps MVIC			
	Baseline	0.025	0.514
	Week 8	0.005	0.641
UNINVOLVED LIMB			
Quadriceps Isokinetic 60°/sec			
	Baseline	0.000	0.908
	Week 8	0.000	0.931
Quadriceps Isokinetic 120°/sec			
	Baseline	0.000	0.885
	Week 8	0.000	0.953
Quadriceps MVIC			
	Baseline	0.000	0.874
	Week 8	0.000	0.897

p value – significance level; r value – correlation coefficient

5.3.2 Quadriceps Femoris Muscle Cross-Sectional Area (CSA)

The coefficient of variation by a single observer for three measurements of CSA was less than 1% for both limbs at each time point. QFM CSA increased by 7.6% (p=0.021) in the involved limb of the NMES group at wk 8 compared to baseline whereas no change was found in the Con group (Figure 5.2). Uninvolved QFM CSA did not change in either group. There was no significant difference in CSA between limbs in either group over the study period. QFM CSA did not correlate with subject age. Greater muscle CSA was strongly associated with both taller subjects, although it did not correlate with BMI. (Table 5.3)

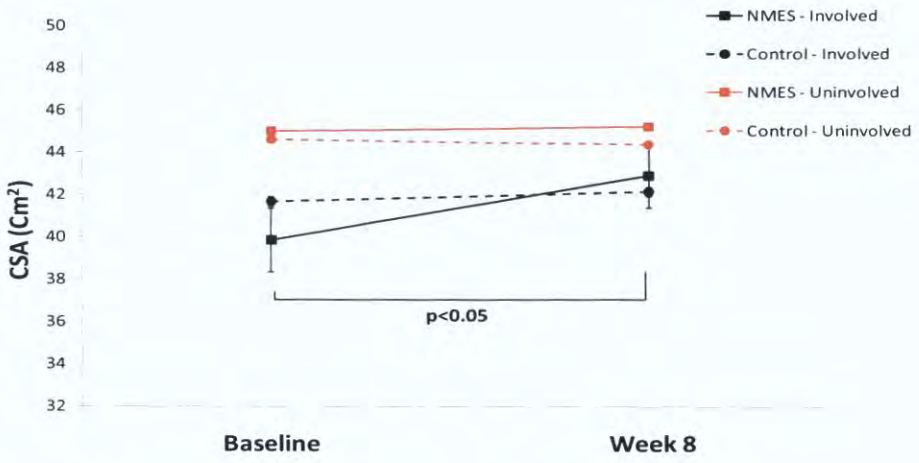


Figure 5.2. Quadriceps femoris muscle cross-sectional area

Table 5.3. Significant correlations between quadriceps femoris cross-sectional area and subject height

Comparison		p value	r value
Involved CSA			
	Baseline	0.001	0.725
	Week 8	0.001	0.725
Uninvolved CSA			
	Baseline	0.000	0.774
	Week 8	0.000	0.784

p value – significance level; r value – correlation coefficient

5.3.3 Quadriceps Muscle Specific Force (F^0)

There was no difference in specific force between groups over the study period. Isokinetic QFM specific force at 60°/sec was greater in both limbs of both groups at week 8 compared to baseline ($p<0.05$) (Table 5.4). At 120°/sec, an increase in specific force was only found in the Con group ($p<0.05$). When specific force was calculated from isometric peak torque [F^0 (0°/sec)], the involved limb increased by 11.7% ($p=0.086$) and 15.4% ($p=0.046$) in the NMES group and Con groups respectively. Improvements in F^0 (0°/sec) were also found in the uninvolved limb of both groups at wk 8 compared to baseline ($p<0.05$). There was no difference in specific force between limbs of the Con group over the study period. Specific

force at 60°/sec was 23.6% greater (p=0.028) in the uninvolved limb of the NMES group compared to the involved limb. By week 8 this difference had reduced to 12.6% (p=0.110).

Table 5.4. Quadriceps femoris muscle specific force

	Time			
	Involved Limb		Uninvolved Limb	
	Baseline	Week 8	Baseline	Week 8
F^0 (60°/sec) (Nm/cm^2)				
NMES	1.4 ± 0.4*	1.7 ± 0.5[a]	1.8 ± 0.7	1.9 ± 0.6[a]
Control	1.6 ± 0.5	1.9 ± 0.6[a]	1.5 ± 0.4	1.9 ± 0.5[a]
F^0 (120°/sec) (Nm/cm^2)				
NMES	1.3 ± 0.3	1.3 ± 0.4*	1.5 ± 0.5	1.6 ± 0.4
Control	1.4 ± 0.4	1.6 ± 0.5[a]	1.3 ± 0.3	1.6 ± 0.4[a]
F^0 (0°/sec) (Nm/cm^2)				
NMES	2.0 ± 0.3	2.2 ± 0.3	2.2 ± 0.8	2.5 ± 0.8[a]
Control	2.2 ± 0.8	2.6 ± 0.7[a]	2.2 ± 0.5	2.4 ± 0.5[a]

Values are means ± SD; [a] p<0.05 vs. baseline; * p<0.05 vs Uninvolved limb
QFM – Quadriceps; MVIC – Maximum Voluntary Isometric Contraction.

Subject age did not correlate with specific force. Subject height was not associated with specific force of the involved limb whereas strong associations (p<0.01) were found with uninvolved limb isokinetic specific force (Table 5.5). There was an inverse correlation between BMI and uninvolved specific force (Table 5.6). Only weak inverse relations were found between BMI and involved specific force when determined from isokinetic peak torque (60°/sec) at baseline (r^2=0.21; p=0.043) and MVIC at wk 8 (r^2=0.22; p=0.039).

Table 5.5 . Significant correlations between uninvolved quadriceps muscle specific force and subject height

Comparison		p value	r value
F^0 (0°/sec)			
	Baseline	0.041	0.464
F^0 (60°/sec)			
	Baseline	0.001	0.757
	Week 8	0.003	0.666
F^0 (120°/sec)			
	Baseline	0.003	0.668
	Week 8	0.001	0.725

p value – significance level; r value – correlation coefficient; F^0 – Specific Force

Table 5.6. Significant correlations between uninvolved quadriceps muscle specific force and subject BMI

Comparison		p value	r value
F^0 (0°/sec)			
	Baseline	0.009	-0.604
	Week 8	0.029	-0.500
F^0 (60°/sec)			
	Baseline	0.011	-0.586
	Week 8	0.006	-0.629
F^0 (120°/sec)			
	Baseline	0.015	-0.557
	Week 8	0.041	-0.464

p value – significance level; r value – correlation coefficient; F^0 – Specific Force

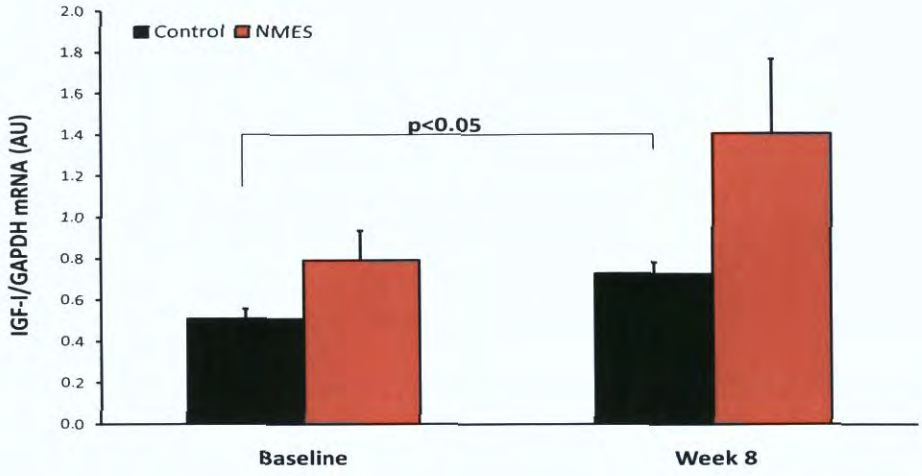
5.3.4 Gene Expression

There was no difference in levels of gene expression between the study groups. IGF-1 expression increased by 78.5% (p=0.110) and 43.1% (p=0.028) in the NMES and Con groups respectively (Figure 5.3). There was no significant change in expression of MAFbx and MURF-1 in either group.

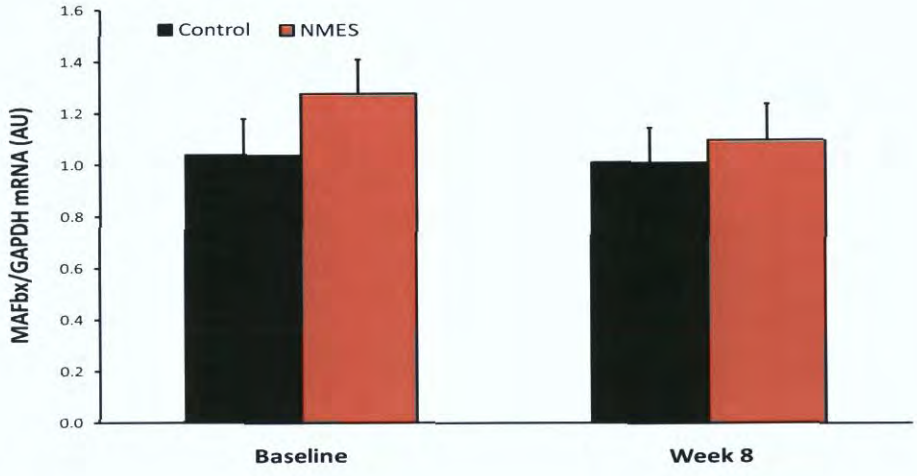
Myosin heavy chain isoform expression did not change in either group over the study period. MHC-I expression decreased by 10.6% (p=0.139) in the NMES group and increased by 25.0% (p=0.173) in the Con group (Figure 5.4). The difference in the percentage change in MHC-I expression between groups was close to statistical significance (p=0.077). MHC-IIx expression decreased by 42.2% (p=0.477) in the NMES group compared to a 14.8% increase (p=0.753) in the Con group.

Subject age was inversely related to MURF-1 expression at baseline (r^2=0.30; p=0.017). Subject height had a strong inverse correlation with MAFbx at baseline (r^2=0.42; p=0.004) while weak inverse relations were found at wk 8 with IGF-1 (r^2=0.25; p=0.028), MURF-1 (r^2=0.20; p=0.049) and MHC-IIx (r^2=0.21; p=0.045). Subject BMI did not correlate with IGF-1, MAFbx or MHC isoform expression, but a direct association was found with MURF-1 at baseline (r^2=0.39; p=0.006) and wk 8 (r^2=0.31; p=0.016).

a) IGF-1



b) MAFbx



c) MURF-1

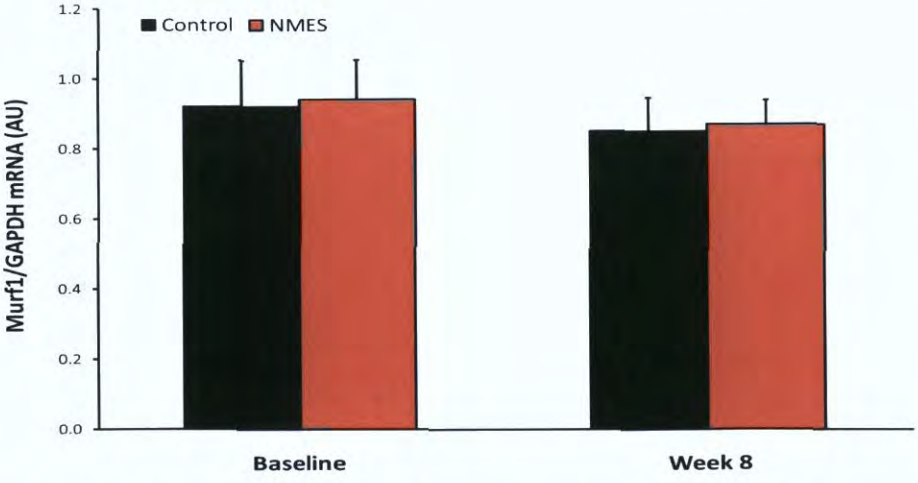
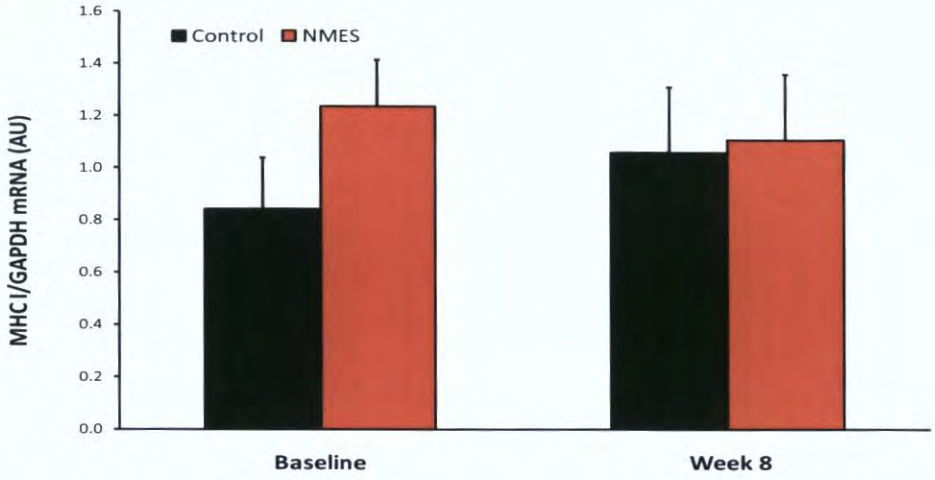
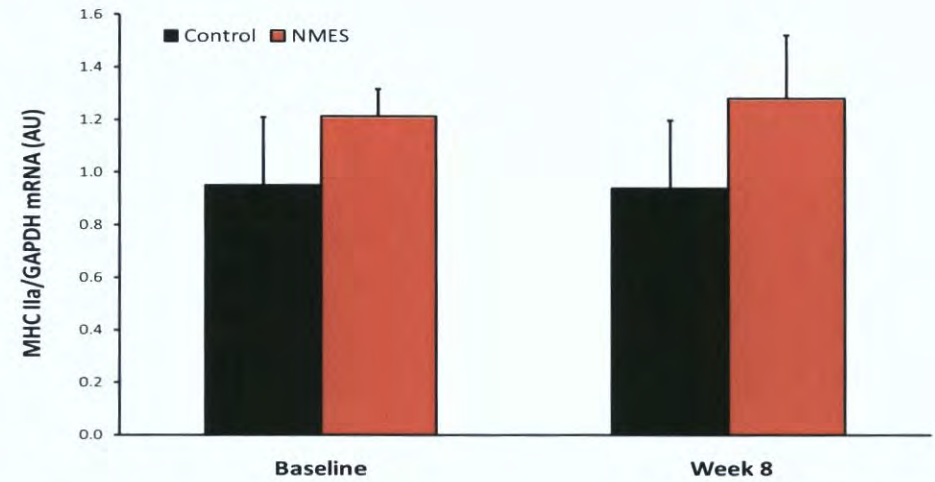


Figure 5.3. Expression of genes associated with muscle anabolism and catabolism

a) MHC-I



b) MHC-IIa



c) MHC-IIx

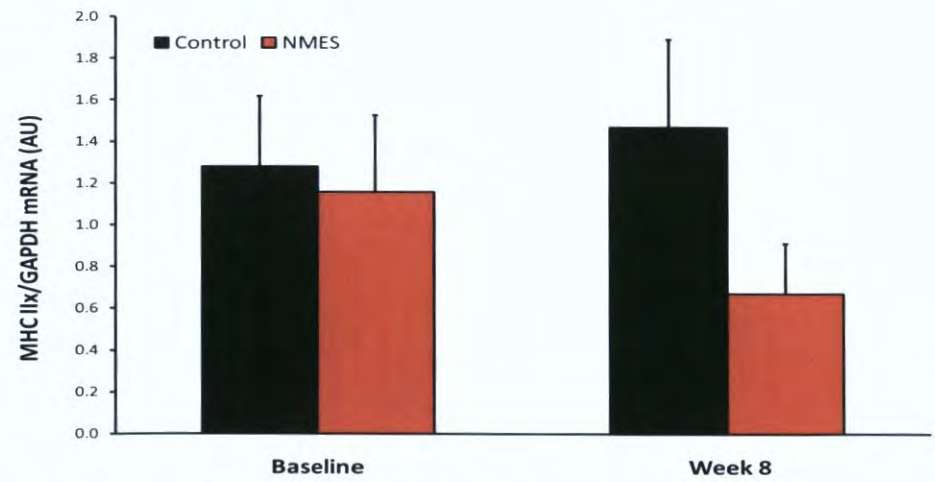


Figure 5.4. Myosin heavy chain gene expression

5.3.5 Significant Correlations Between Outcome Measures

QFM CSA strongly related to muscle strength in both limbs (Table 5.7). There was no relation between percentage change in muscle strength and percentage change in CSA. Changes in QFM isokinetic peak torque at 60°/sec strongly correlated with changes in isokinetic strength at 120°/sec (r^2=0.37; p=0.008), although neither correlated with changes in MVIC.

Improvements in isokinetic peak torque at 120°/sec strongly related to increased MHC-IIa expression (r^2=0.49; p=0.002) and moderately related to increased expression of MAFbx (r^2=0.20; p=0.048) and MURF-1 (r^2=0.33; p=0.012). There was an inverse relation between QFM CSA and MAFbx at baseline (r^2=0.28; p=0.022). Changes in QFM CSA did not relate to changes in gene expression. There was a weak association between changes in F^0 (60°/sec) and MHC-I (r^2=0.20; p=0.048). Changes in specific force at 120°/sec related to changes in MHC-IIa (r^2=0.36; p=0.009), MAFbx (r^2=0.21; p=0.045) and MURF-1 (r^2=0.28; p=0.021).

There were several significant correlations between the studied genes (Table 5.8). At baseline, MHC-IIa was inversely related to MHC-IIx and directly related to MHC-I expression. IGF-1 also had a strong association with MHC-1 at baseline. At wk 8, there was an inverse relation between MAFbx and MHC-IIx while MHC-1 strongly related to MAFbx and MURF-1 expression. Changes in MURF-1 expression over the study period were strongly associated with changes in both MAFbx (r^2=0.41; p=0.005) and MHC-IIa expression (r^2=0.39; p=0.006).

Table 5.7. Significant correlations between quadriceps muscle CSA and strength

Comparison		p value	r value
Involved Limb			
Quadriceps Isokinetic 60°/sec			
	Baseline	0.005	0.636
	Week 8	0.004	0.650
Quadriceps Isokinetic 120°/sec			
	Baseline	0.001	0.711
	Week 8	0.002	0.704
Quadriceps MVIC			
	Baseline	0.005	0.643
	Week 8	0.000	0.782
Uninvolved Limb			
Quadriceps Isokinetic 60°/sec			
	Baseline	0.000	0.843
	Week 8	0.000	0.793
Quadriceps Isokinetic 120°/sec			
	Baseline	0.000	0.843
	Week 8	0.000	0.818
Quadriceps MVIC			
	Baseline	0.001	0.736
	Week 8	0.000	0.768

p value – significance level; *r* value – correlation coefficient

Table 5.8. Significant correlations between individual genes

Comparison		p value	r value
IGF-1 and MAFbx			
	Baseline	0.033	0.486
IGF-1 and MHC-1			
	Baseline	0.005	0.646
MAFbx and MURF-1			
	Baseline	0.037	0.475
	Week 8	0.043	0.457
MAFbx and MHC-1			
	Baseline	0.020	0.536
	Week 8	0.006	0.625
MAFbx and MHC-IIx			
	Week 8	0.032	-0.490
MURF-1 and MHC-1			
	Week 8	0.002	0.696
MURF-1 and MHC-IIa			
	Week 8	0.045	0.454
MHC-1 and MHC-IIa			
	Baseline	0.008	0.611
MHC-IIa and MHC-IIx			
	Baseline	0.007	-0.618
	Week 8	0.006	-0.633

p value – significance level; r value – correlation coefficient

5.4 DISCUSSION

This study compared the effects of an eight week NMES program and standard preoperative care using subjects with end-stage knee OA requiring TKA. Both groups significantly increased QFM strength and muscle specific force. Quadriceps muscle mass (CSA) increased in the involved limb of the NMES group only. Changes in MHC gene expression indicated a fast to slow isoform shift. IGF-1 expression increased in both groups whereas no change was found in transcription levels of MAFbx or MURF-1.

There were significant strength improvements in both limbs of both groups. It would seem that NMES is no better than advice on muscle strengthening exercise. However, motor learning of the dynametric testing protocol may have had an effect, masking actual strength gain. This would also explain strength increases seen in the uninvolved limb of both groups. Quadriceps strength of the involved limb was weaker than the uninvolved limb at baseline but by the end of the study there was no significant difference. Therefore, NMES did exert a substantial training effect

For the whole cohort at baseline, QFM CSA of the involved limb was 9.1% lower than the uninvolved side. Previous studies that compared muscle CSA between involved and uninvolved limbs in subjects with knee OA have reported differences of up to 12%.(154;169) QFM CSA increased in the involved limb of the NMES group only, and to a lesser degree than muscle strength. Exercise training of elderly adults has demonstrated a similar pattern with greater gains in strength than CSA.(112) This must represent improvement in muscle activation and is supported by the increases in muscle specific force and a lack of correlation between changes in QFM strength and CSA seen in this study.

Gondin et al studied the effects of 8 weeks NMES on quadriceps strength, CSA, and activation deficits using healthy young subjects.(223) Neural adaptation explained initial strength improvements whereas increases in CSA accounted for increases in strength beyond 4 weeks of training. Greater strength gains were produced by subjects who had larger voluntary

activation deficits. This is important since subjects with knee OA have greater activation deficits than healthy counterparts and, as we have found, can respond favourably to NMES training.

There is little data available reporting the effects of NMES on MHC mRNA expression. We expected an increase in type II MHC mRNA expression since fast motor units are activated to a greater extent than normally occurs with exercise training. MHC IIx mRNA expression decreased by 42% in the NMES group whereas MHC I and IIa mRNA expression did not change, indicating a fast to slow shift. A similar pattern has been seen with exercise training. A week-long endurance training program decreased MHC IIx mRNA but, like us, found no effect on MHC I or IIa isoform expression.(128) A longer program decreased MHC IIx mRNA by 50% with corresponding increases in MHC I and IIa mRNA of 63% and 99% respectively.(18) Therefore, both NMES and volitional exercise induce a fast to slow MHC mRNA shift with changes in MHC IIx occurring first in the transition between MHC isoforms. There may be variations in the time-course of transcription regulatory mechanisms such that longer programs are necessary to provide sufficient stimulus for changes in type IIa and type I mRNA isoforms.

Our results supports research from Maffiuletti et al on skeletal muscle MHC protein content where NMES decreased levels of MHC IIx by 28% and increased MHC IIa by 22%.(220) A similar phenotype shift has been found in subjects with spinal cord injury following long-term NMES.(291) Such plasticity of MHC isoform protein has been frequently reported in response to volitional exercise programs.(68;126;127)

IGF-1 expression increased in both study groups although it was to a greater extent in the NMES group (79% versus 43%). As this is the first study to assess the effects of NMES on IGF expression, comparisons can only be made with exercise training programs. Five weeks resistance training of healthy elderly men increased IGF-1 levels by 68-75% which compares favourably with our results.(129) An association between IGF-1 expression and age has also been described however disuse and gene down-regulation would explain the lack of such a

finding in our cohort.(84) There was no change in either of the muscle-specific E3 ubiquitin ligases associated with muscle atrophy (MURF-1 and MAFbx). However, there was a strong relation between changes in MURF-1 and MAFbx expression. This is unsurprising as both would be expect to change in the presence or absence of a stimulus given they are connected to a common catabolic pathway.

Changes in MHC IIa mRNA were related to changes in MURF-1 expression. In addition MURF-1 and MAFbx were associated with higher levels of MHC I and lower levels of MHC IIx at the end of the study. These effects seem paradoxical as it indicates that genes associated with muscle atrophy are increased in subjects who have evidence of a training effect (fast to slow MHC shift). It may be that MURF-1 and MAFbx are increased physiologically rather than pathologically. Subjects who responded to NMES also increased their muscle mass and so the mechanisms involved in muscle cell homeostasis may all be upregulated to maintain normal cell turnover.

Muscle immobilisation and disuse result in a switch from a slow to fast muscle fibre type, whereas preferential denervation of type II fibres due to motor unit remodelling as part of the aging process produce a slower phenotype.(33) Differences in baseline MHC mRNA reflect variable effects of these two processes in our cohort and therefore, the lack of significant correlation with subject age.

While radiological assessments are considered sensitive for changes in muscle CSA, information is taken from only one portion of the thigh. It is assumed this reflects whole muscle volume. Narici et al reported non-uniform changes in the quadriceps muscle following training in young healthy subjects.(292) Since most studies use MRI to assess training effects on quadriceps CSA, our data can be considered comparable.

An 8 week NMES program increases muscle strength primarily through neurological adaptation, although significant muscle hypertrophy also occurs. There is evidence of a transition from a fast to slow MHC genotype which appears to be similar to that which occurs

with exercise training. IGF-1 expression increases dramatically in response to NMES. Changes in the muscle-specific E3 ubiquitin ligases are less pronounced although some patterns of association with MHC expression have been demonstrated and require further study.

Chapter VI

Efficacy of a Prehabilitation Program in Total Knee Arthroplasty using Neuromuscular Electrical Stimulation

6.1 INTRODUCTION

Patients with knee OA have significant quadriceps femoris muscle (QFM) weakness and functional disability primarily due to muscle activation failure.(163) Lower levels of QFM strength and function prior to total knee arthroplasty (TKA) result in lower functional endpoints after surgery.(293) In addition, declines in muscle strength following TKA, mainly due to incomplete muscle activation, can further impact negatively on functional recovery.(260;278;294)

Therapeutic exercise improves function in patients with arthritis of the knee, yet to our knowledge only 5 studies have assessed the impact of prehabilitation (preoperative strengthening) in TKA.(295-301) Reported benefits include improved preoperative strength by up to 20%, reduced postoperative length of stay and a greater likelihood of discharge home rather than to a rehabilitation facility.(297;300) Supervised prehabilitation programs are expensive, labour intensive requiring trained personnel and can pose difficultly for patients regarding transportation and time commitments.(297;299;300)

Neuromuscular electrical stimulation (NMES) causes muscle contraction by applying transcutaneous current to the neuromuscular junction and surrounding muscle fibres. A 12 week home-based NMES program produced a 9% increase in quadriceps strength with concomitant functional improvement in subjects with knee OA.(247) Durmus et al also found it to be as effective as exercise therapy in osteoarthritis.(248) As a rehabilitation adjunct post-TKA, NMES has helped to reduce postoperative stay while significantly improving walking speed and extensor lag.(251;252) These gains may be attributable to decreased neuromuscular inhibition permitting greater voluntary muscle activation.(276)

Home-based NMES training could potentially overcome many of the difficulties associated with preoperative supervised exercise programs. The primary objective of this study was to evaluate objective functional recovery from 6 to 12 weeks post-TKA using timed walk, stair climb and chair rise tests. In addition, we wanted to assess preoperative and postoperative changes in muscle strength, quadriceps CSA, subjective outcome instruments and anthropometric measures.

6.2 PATIENTS AND METHODS

6.2.1. Patients

Men and women between 45 and 80 years who were undergoing TKA for primary knee OA were recruited from an orthopaedic preoperative assessment clinic between July and October 2007. Subjects were allocated to either intervention (NMES) or control (Con) groups using block randomisation. Patients received individual counselling before enrolment to ensure they understood the nature of the study after which informed consent was obtained.

Seventeen patients enrolled in the study. One patient suffered a postoperative myocardial infarction and another had surgery postponed for management of an unrelated condition. One control subject withdrew due to marked preoperative clinical deterioration. Baseline demographics for the 14 patients who completed the study are presented in table 6.1. There was no statistical difference between groups in any parameter.

Table 6.1. Baseline characteristics

	Group	
	NMES	**Control**
Male: Female	3 : 6	1: 4
Age (y)	64.4 ± 8.0	63.2 ± 11.4
Height (cm)	161.6 ± 13.9	155.8 ± 5.3
Weight (kg)	80.3 ± 13.6	79.5 ± 14.6
BMI (kg/m^2)	30.7 ± 3.0	32.8 ± 6.3
Walking Dsitance (m)	1238.9 ± 1349.5	750.0 ± 691.0
Walking Time (min)	22.8 ± 22.6	17.0 ± 10.4

Values are means ± SD; BMI: Body Mass Index

6.2.2 Overview

The study was 20 weeks in duration; 8 weeks preoperative and 12 weeks postoperative. The intervention group received 8 weeks of preoperative unsupervised, home-based NMES training applied unilaterally to the QFM of the affected side. Subjects assigned to the Con group received standard preoperative care including advice from a physiotherapist on muscle strengthening exercises. All patients received standard postoperative rehabilitation.

Muscle strength, functional and anthropometric assessments were performed and questionnaires administered at baseline and week 8 preoperatively, and at week 6 and week 12 post-TKA. Muscle cross-sectional area (CSA) evaluations were performed at baseline and week 8 preoperatively, and at week 12 postoperatively. One patient in the NMES group did not have preoperative imaging, and was excluded from the CSA analysis.

6.2.3 Intervention – Neuromuscular Electrical Stimulation

The stimulator (KneeHAB II, Bio-Medical Research, Galway, Ireland) was used for 20 min·day^{-1} on alternate days during the initial two weeks to familiarise the subjects. Patients were encouraged to increase the stimulation intensity to their maximum tolerated level during each session. The training program involved a single 20 min session·day^{-1}, 5 days·wk^{-1} for 6 weeks preoperatively. Patients were encouraged to use the stimulator at their highest tolerated intensity. They were instructed to sit with their knees flexed to 60°, feet flat on the ground and their toes against a wall to permit isometric training. A training logbook was provided to subjects in the NMES group to record the duration of each session. In addition and unknown to the patients, the stimulator also recorded total usage.

6.2.4 Evaluation Protocols

6.2.4.1 Muscle Strength

A Biodex dynamometer (Biodex Medical Instruments, Shirley, NY) determined quadriceps (QFM) and hamstring (HS) peak torque (Nm) of both limbs as previously described

(Chapter III). Concentric isokinetic QFM (extension) and HS (flexion) strength were assessed at two angular velocities: 60°/sec and 120°/sec. Maximum voluntary isometric contraction (MVIC) peak torque was assessed with the knee flexed at 60 degrees. The greatest force generated in each test was recorded. Standardised verbal coaching was given by a research assistant blinded to each participant's group assignment.

6.2.4.2 Quadriceps Femoris Muscle Cross-sectional area

Magnetic Resonance Imaging (MRI) determined QFM cross-sectional area (CSA) of both thighs using a Gyroscan Intera 1.5T MRI scanner (Philips Medical Systems, Holland). Images were taken at the level of the mid-thigh using the greater trochanter and lateral knee joint line as anatomical markers. Manual planimetry outlined the QFM the region of interest and CSA (cm^2) was automatically calculated.

6.2.4.3 Functional Evaluation

A timed chair-rise test (TCT), 25-metre timed walking test (TWT), and a timed stair climbing test (TST) were used to assess functional capacity. A straight back chair with adjustable leg height was used for the TCT. Subjects sat with their knees flexed at 90° and their arms folded across their chest. The time taken to complete 3 full cycles was recorded. The 25-metre TWT was performed in an indoor hallway with subjects allowed to stop or use a mobility aid if required. The TST was performed on an indoor stairwell with 11 steps (18 cm rise, 30 cm depth). Patients were asked to try and refrain from using the handrails during the test, and the time taken to ascend, turn, and descend was recorded. Each test was performed 3 times and the fastest time recorded. Assessments were performed in the same order for each participant.

6.2.4.4 Anthropometric Evaluation

Knee range of motion (ROM, degrees) and extension lag (degrees) were determined using a goniometer. Thigh circumference (cm) was assessed at the mid-point between the greater trochanter and the lateral joint line to correspond with MRI CSA evaluation.

6.2.4.5 Self-Report Outcome Measures

Individual perception of disability was assessed using three subjective scoring instruments. The Western Ontario McMaster Osteoarthritis Index (WOMAC) is sub-categorized into pain (0-20), stiffness (0-8), and function (0-68) where a lower score in each is considered better. The Medical Outcome Study short form 36 (SF-36) evaluates general health using a 36 item questionnaire with component scores given for physical and mental health. Both are scored from 0 to 100 with a higher score indicating better health. The Oxford knee score assesses the impact of knee pain on activities of daily living using a 12 item outcome instrument. Each item is scored on a 5 point scale providing summated scores from 12 (best) to 60 (worst).

6.2.5 Surgical Technique and Postoperative Management

All TKA's were performed by one of two consultant orthopaedic surgeons under tourniquet control. A midline incision was made with a standard medial parapatellar approach to the joint. A quadriceps snip was not performed in any knee. The posterior cruciate ligament (PCL) was sacrificed in all cases with insertion of either the Scorpio PS knee system (Stryker, Limerick, Ireland), or the Triathlon knee system (Stryker, Limerick, Ireland) depending on the operative surgeon's preference. All operations were performed under spinal anaesthesia and patients received PCA or epidural analgesia in the immediate postoperative period. A standardised rehabilitation program was commenced on the first postoperative day. The length of postoperative hospital admission and discharge location (home or rehabilitation facility) were recorded.

6.2.6 Statistical Analysis

Independent t-tests were used to evaluate potential group differences for age, height, weight, and walking time. The non-parametric Friedman test was used to compare within group differences in strength, CSA, function, clinical evaluations, and subjective outcomes. Post hoc analyses was performed using the Wilcoxan signed ranks tests with Bonferroni adjustment for multiple comparisons. Between group comparisons were performed using the Mann Whitney test. The relation between selected parameters was determined using Spearman rho correlations. Statistical significance was set at p=0.05. Statistical tests were performed using the Statistical Package for Social Sciences version 15.0 (SPSS, Inc., Chicago, IL).

6.3 RESULTS

6.3.1 Compliance

Overall patient reported compliance was 99.4% (range, 97.1-100%) and stimulator-recorded compliance was 99.0% (range, 77.2-114.8%). Stimulator recorded compliance was more than 97% in all but one male subject who reduced his usage between wks 5 and 8 by 52.6%. This was inconsistent with his logbook compliance (97.7%) over the same period.

6.3.2 Muscle Strength

There was no difference in strength between groups at any time point. At baseline, involved QFM peak torque was lower compared to the uninvolved limb in the NMES group (Figure 6.1): isokinetic 60°/sec, 29.9% ($p=0.015$); isokinetic 120°/sec, 22.1% ($p=0.066$); isometric, 19.4% ($p=0.086$). Isokinetic HS strength at 60°/sec was also lower (19.7%; $p=0.051$) in the involved limb of the NMES group. There was no difference in QFM or HS strength between limbs of the Con group at baseline.

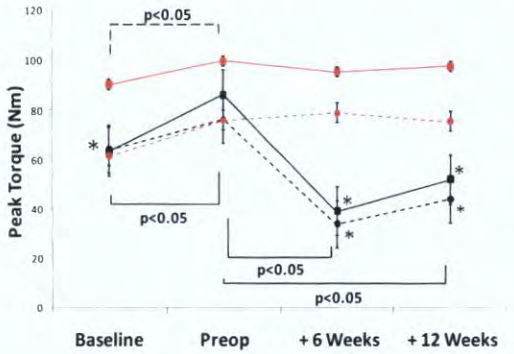
Preoperatively, QFM isokinetic peak torque at 60°/sec and isometric strength increased by 36.1% ($p=0.008$) and 27.8% ($p=0.021$; alpha level=0.013) respectively in the involved limb of the NMES group compared to baseline. Isokinetic peak torque at 120°/sec did not change. Involved HS strength of the NMES group increased by 48.7% ($p=0.011$) and 46.8% ($p=0.008$) at 60°/sec and 120°/sec respectively. Although not statistically significant, preoperative HS isokinetic strength at 120°/sec was greater in the involved limb (16.4%, $p=0.208$). There was no difference in QFM or HS strength between limbs in either group preoperatively ($p>0.05$).

When compared to preoperative muscle strength, the decrease in involved QFM peak torque was similar in both groups at 6 weeks post-TKA: isokinetic 60°/sec (54.4% – 55.8%), isokinetic 120°/sec (48.9% – 52.3%) and isometric (51.8% - 52.7%). These declines were only significant in the NMES group ($p<0.05$). Declines in HS peak torque were not statistically

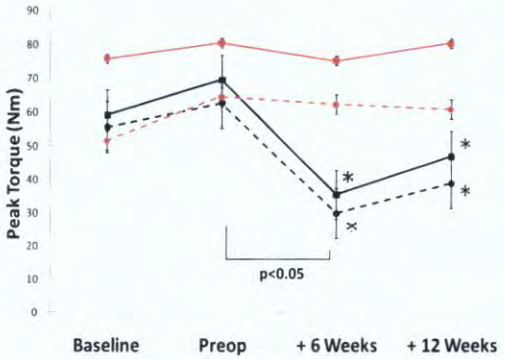
significant in either group. At 6 wks post-TKA, the operated limb was significantly weaker ($p<0.05$) compared to the contralateral limb; post-TKA; QFM: 52.0% - 58.9%; HS: 32.4% - 58.1%.

From 6 to 12 wks post-TKA, an increase in QFM peak torque was only found in the NMES group when determined isometrically (53.3%, $p=0.011$). Improvements in isokinetic QFM strength were close to statistical significance; 60°/sec, 33.0% ($p=0.021$ [alpha level=0.013]); 120°/sec, 32.3% ($p=0.021$ [alpha level=0.013]). There was no change in QFM strength in the control group or HS strength in either group over this period. At 12 wks post-TKA, the uninvolved limb was stronger than the operated limb in both groups ($p<0.05$): quadriceps: 36.1% - 46.6% hamstring: 30.7% - 44.4%.

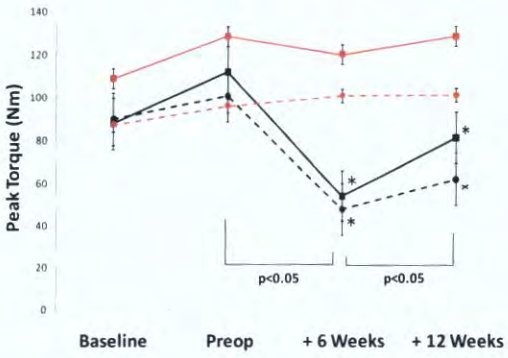
a) Quadriceps isokinetic peak torque – 60°/sec



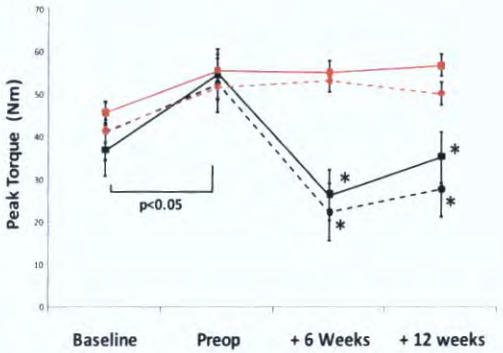
b) Quadriceps isokinetic peak torque – 120°/sec



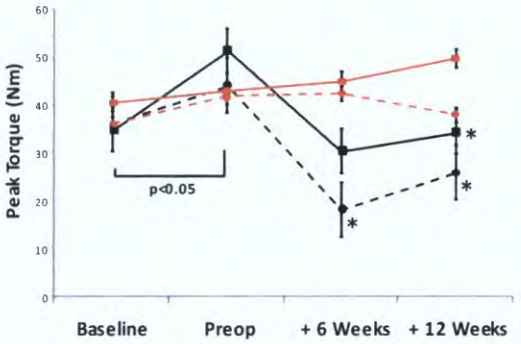
c) Quadriceps isometric peak torque



d) Hamstring isokinetic peak torque – 60°/sec



e) Hamstring isokinetic peak torque – 120°/sec



* p<o.o5 vs. Uninvolved

NMES - Involved

Control - Involved

NMES - Uninvolved

Control - Uninvolved

Figure 6.1. Isokinetic and isometric peak torque

6.3.3 Quadriceps Muscle Cross-Sectional Area

Quadriceps CSA of the uninvolved limb did not change in either group over the study period and there was no significant difference between groups at any time point (Figure 6.2). Preoperatively, there was a trend towards an increase in QFM CSA of the involved limb in the NMES group (7.4%, p=0.036 [alpha level=0.017]). Although involved QFM CSA decreased by approximately 10% postoperatively in both groups, it was only significant in the NMES group (p=0.012). Compared to baseline, involved quadriceps muscle CSA at 12 weeks post-TKA had decreased by 3.7% and 12.1% in the NMES and Con groups respectively, although neither was statistically significant. When compared to the uninvolved limb, QFM CSA of the involved limb at 12 weeks post-TKA was 13.4% lower (p=0.08) in the control group. The coefficient of variation (intraobserver variability) in determining CSA from MR imaging was less than 1% in all cases.

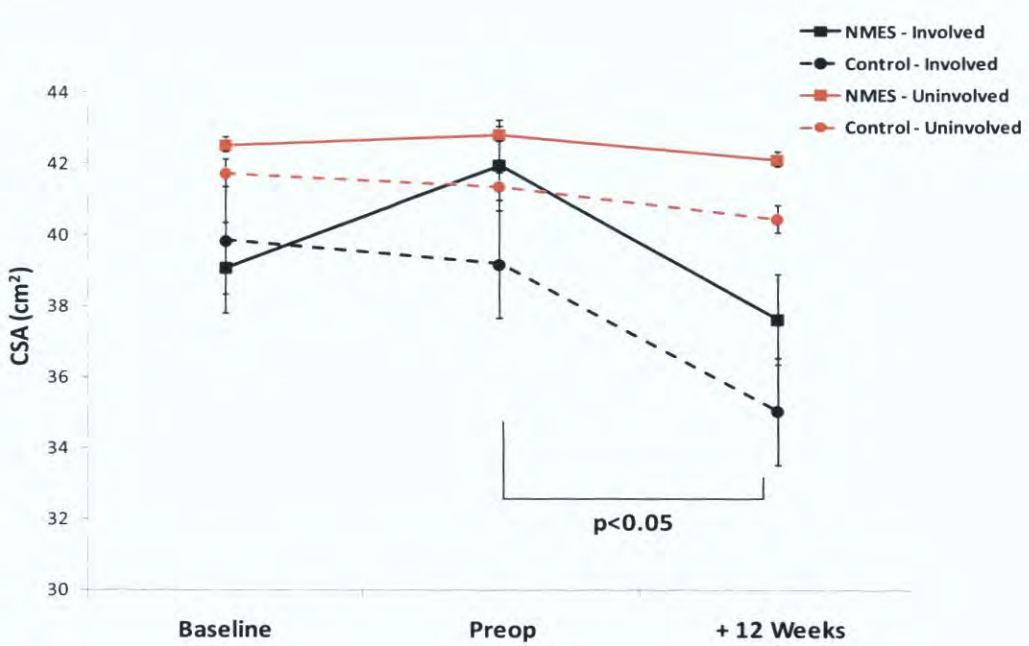


Figure 6.2 Quadriceps muscle cross-sectional area

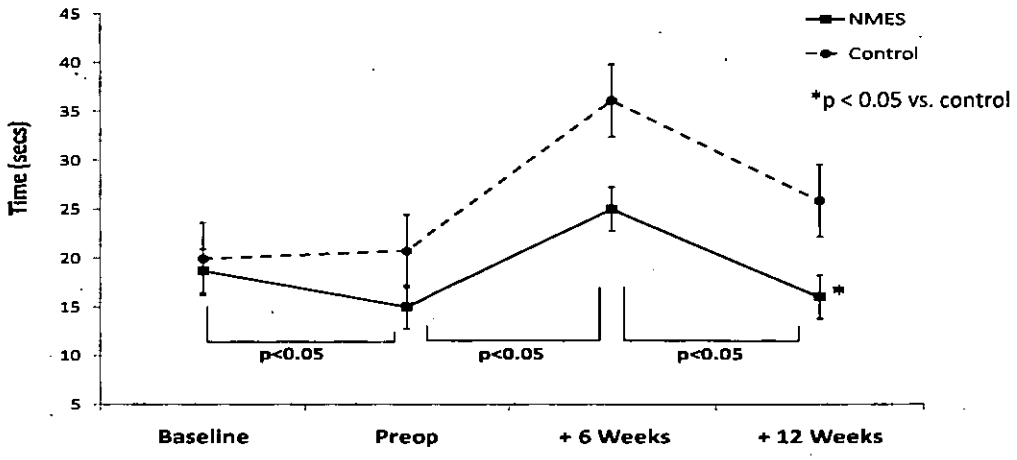
6.3.4 Objective Function

Performance in objective function did not change in the Con group preoperatively (Figure 6.3). There were improvements in the 25-metre timed walk test (TWT) (9%, p=0.008), timed stair-climb test (TST) (19.7%, p=0.008), and timed chair-rise test (TCT) (34.2%, p=0.008) in the NMES group compared to baseline. Performance in the TCT was 25.9% better (p=0.019) in the NMES group than the Con group preoperatively.

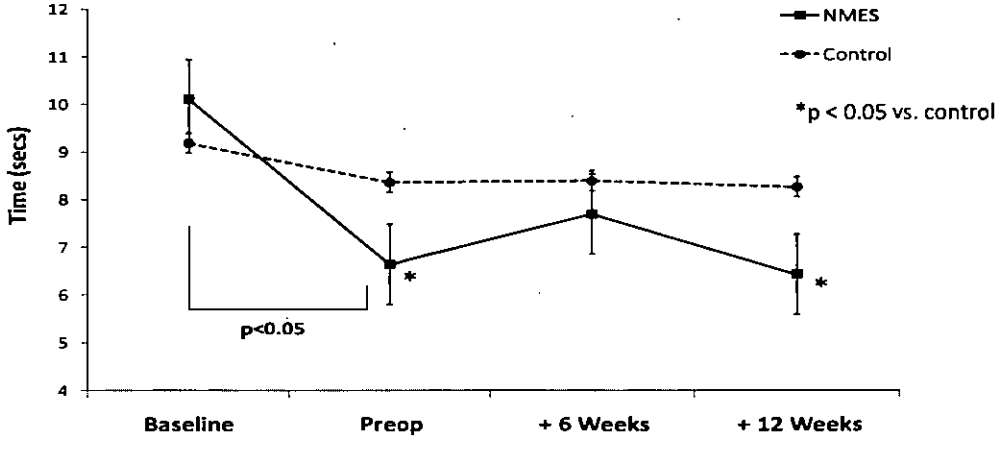
The time required to complete the TST and TWT increased by 66.8% (p=0.008) and 35.5% (p=0.008) respectively in the NMES group at 6 wks postoperatively compared to preoperative values. Although there were similar declines in the Con group (TST, 74.2%; TWT, 25.5%), these did not achieve statistical significance. Compared to preoperative performance, the time taken to perform the TCT did not change in either group when assessed at 6 wks.

All measures of functional capacity were better in the NMES group at wk 12 than wk 6 post-TKA (TWT: 22.9% [p=0.008]; TST: 36.8% [p=0.008]; TCT: 16.4% [p=0.015]). The Con group did not improve significantly in any test. Performance was better by the NMES group compared to the Con group in the TST (61.6%, p=0.029) and TCT (34.2%, p=0.019) at 12 wks post-TKA.

a) Timed stair-climb test (TST)



b) Timed chair-rise test (TCT)



c) 25-metre timed walk test (TWT)

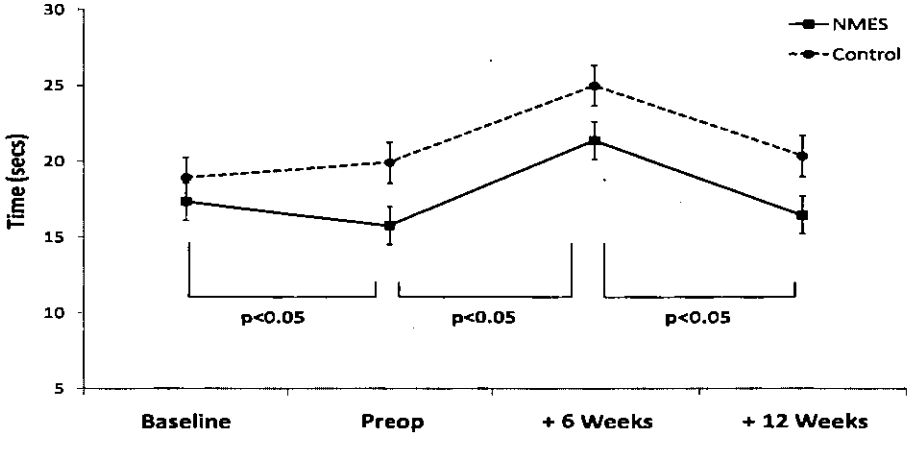


Figure 6.3. Objective function

6.3.5 Anthropometric Evaluation

Knee ROM and thigh circumference were similar in both groups and neither changed significantly over the study period (Table 6.2). Extension lag at baseline was greater (p=0.013) in the NMES group than the Con group (Figure 6.4). Despite improving by 33% preoperatively in the NMES group, extension lag was still greater (p=0.022) compared to the control group. At 6 wks post TKA there was a greater (p=0.011) extension lag in the NMES group compared to preoperative values. There was no difference in extension lag between the study groups post-TKA.

Table 6.2. Clinical evaluation

	Time			
	Baseline	**Preoperative**	**+ 6 Weeks**	**+ 12 Weeks**
Extension Lag (degrees)				
NMES	11.4 ± 7.7 *	7.7 ± 5.3 *	15.4 ± 4.5 [a]	12.7 ± 6.3
Control	0.0 ± 0.0	0.4 ± 0.9	15.2 ± 8.7	10.8 ± 8.0
Range of Motion (degrees)				
NMES	106.2 ± 17.8	108.2 ± 18.6	91.6 ± 17.2	98.6 ± 16.6
Control	120.4 ± 12.9	119.4 ± 13.2	91.0 ± 19.9	96.6 ± 17.4
Thigh Circumference (cm)				
NMES	48.0 ± 3.5	48.2 ± 2.8	49.2 ± 2.9	47.7 ± 2.5
Control	51.3 ± 5.4	51.7 ± 5.8	51.2 ± 6.0	51.6 ± 5.7

Values are means ± SD ; *p < 0.05 vs. control; [a]<0.05 vs. Preoperative

6.3.6 Self-Report Outcome Measures

WOMAC function and stiffness scores of the NMES group and all scores of the Con group did not change significantly over the study period (Figure 6.5). There was no difference between groups in any outcome measure. Postoperatively, SF-36-physical health (p=0.008), SF-36-mental health (p=0.011) and oxford knee scores (p=0.012) improved in the NMES group at wk 12 compared to wk 6. Compared to preoperative values, the NMES groups reported improvements at wk 12 in their WOMAC pain (p=0.012) SF-36-mental health (p=0.011), and oxford knee scores (p=0.011).

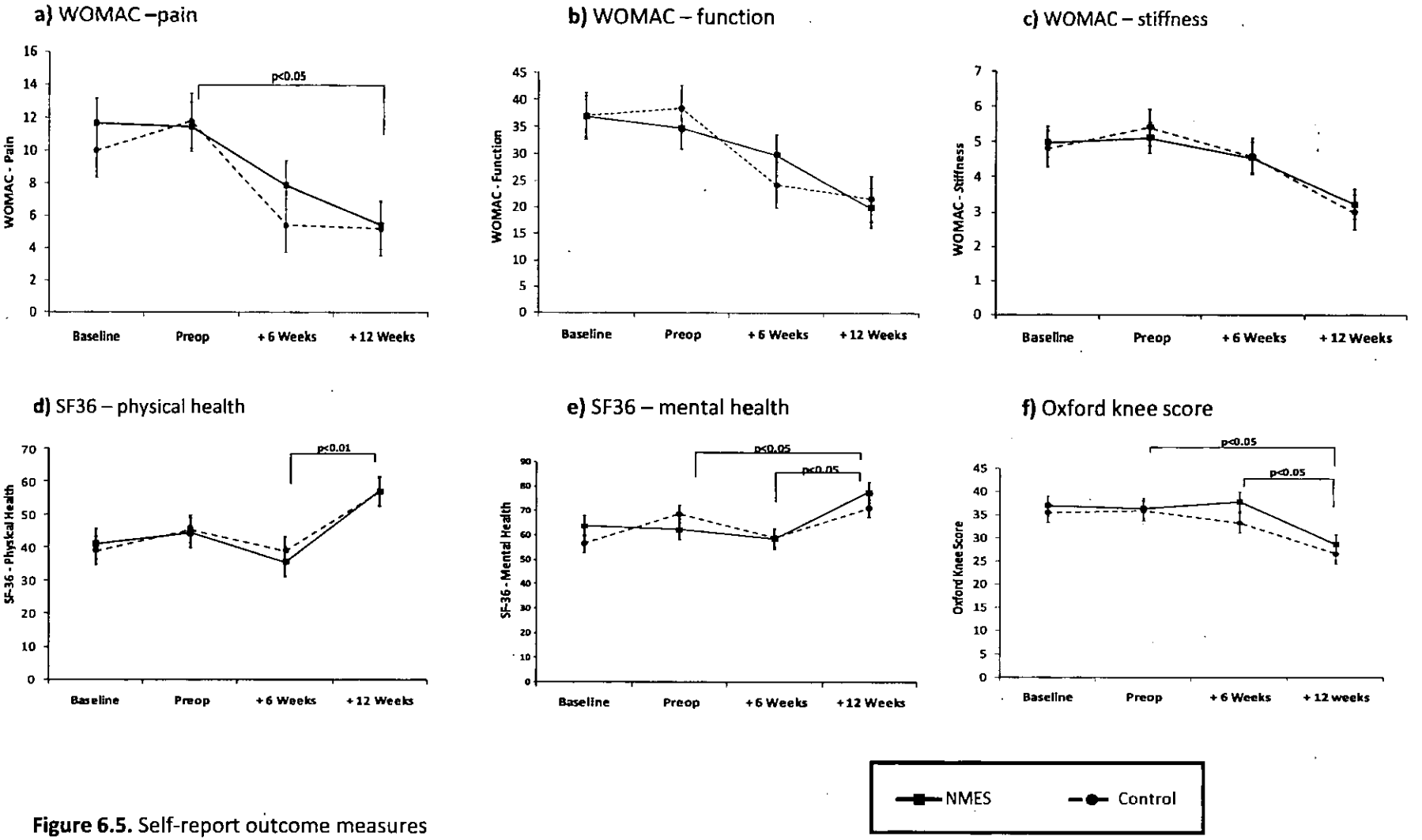


Figure 6.5. Self-report outcome measures

6.3.7 Early Postoperative Rehabilitation

Although not statistically significant, the NMES group had a lower length of postoperative hospitalisation (8.1 days versus 8.8 days, p=0.944). Two of nine patients in the NMES group and one of five patients in the control group required transfer to a rehabilitation facility on discharge.

6.3.8 Significant Correlations Between Outcome Measures

There were significant relations between QFM strength and QFM CSA at all time points in both limbs (Table 6.3). All measures of objective functional capacity correlated strongly ($p<0.01$) with each other preoperatively (Table 6.4). Postoperatively, strong associations were only found between the TST and TWT. In general, performance of the TWT and TST correlated strongly with QFM and HS peak torque of both limbs over the study period (Tables 6.5 & 6.6). Performance at the TCT correlated strongly with muscle strength of the uninvolved limb at preoperative assessments only (Table 6.6), and the few relations found between TCT and involved limb strength were generally weak ($p>0.03$). There were limited relations found between objective functional capacity and subjective outcome scores (Table 6.7).

Table 6.3. Significant correlations between quadriceps muscle strength and cross-sectional area

Comparison		p value	r value
Involved Isokinetic 60°/sec			
	Baseline	0.013	0.615
	Preoperative	0.002	0.731
	+ 12 Weeks	0.008	0.654
Involved Isokinetic 120°/sec			
	Baseline	0.011	0.626
	Preoperative	0.003	0.715
	+ 12 Weeks	0.001	0.780
Involved MVIC			
	Baseline	0.017	0.588
	Preoperative	0.001	0.753
	+ 12 Weeks	0.014	0.604
Uninvolved Isokinetic 60°/sec			
	Baseline	0.001	0.786
	Preoperative	0.003	0.709
	+ 12 Weeks	0.010	0.632
Uninvolved Isokinetic 120°/sec			
	Baseline	0.001	0.764
	Preoperative	0.003	0.725
	+ 12 Weeks	0.010	0.632
Uninvolved MVIC			
	Baseline	0.010	0.632
	Preoperative	0.008	0.648
	+ 12 Weeks	0.001	0.758

p value – significance level; r value – correlation coefficient
CSA = Cross-sectional area

Table 6.4. Correlations between objective functional measures

Comparison		p value	r value
TWT and TST			
	Baseline	0.000	0.877
	Preoperative	0.000	0.873
	Week 6	0.000	0.925
	Week 12	0.000	0.851
TWT and TCT			
	Baseline	0.003	0.691
	Preoperative	0.001	0.754
	Week 12	0.021	0.547
TCT and TST			
	Baseline	0.007	0.642
	Preoperative	0.000	0.842
	Week 6	0.033	0.503
	Week 12	0.025	0.534

p value – significance level; r value – correlation coefficient
TWT = Timed walk test / TST = Timed stair-climb test / TCT = Timed chair-rise test

Table 6.5. Correlations between involved muscle strength and objective function

Comparison		p value	r value
Timed Walk Test (TWT)			
Quadriceps Isokinetic 60°/sec			
	Baseline	0.007	-0.635
	+ 6 Weeks	0.005	-0.662
	+ 12 Weeks	0.004	-0.670
Quadriceps Isokinetic 120°/sec			
	Baseline	0.003	-0.692
	+ 6 Weeks	0.012	-0.596
	+ 12 Weeks	0.003	-0.701
Quadriceps MVIC			
	Baseline	0.003	-0.688
	+ 6 Weeks	0.003	-0.697
	+ 12 Weeks	0.005	-0.662
Hamstring Isokinetic 60°/sec			
	Baseline	0.008	-0.626
	+ 6 Weeks	0.008	-0.631
	+ 12 Weeks	0.004	-0.679
Hamstring Isokinetic 120°/sec			
	Baseline	0.002	-0.705
	Preoperative	0.043	-0.475
	+ 6 Weeks	0.000	-0.873
	+ 12 Weeks	0.000	-0.789
Timed Stair-Climb Test (TST)			
Quadriceps Isokinetic 60°/sec			
	Baseline	0.008	-0.631
	Preoperative	0.016	-0.574
	+ 6 Weeks	0.014	-0.587
	+ 12 Weeks	0.002	-0.723
Quadriceps Isokinetic 120°/sec			
	Baseline	0.003	-0.697
	Preoperative	0.006	-0.647
	+ 6 Weeks	0.031	-0.512
	+ 12 Weeks	0.002	-0.727
Quadriceps MVIC			
	Baseline	0.008	-0.626
	Preoperative	0.020	-0.552
	+ 6 Weeks	0.009	-0.618
	+ 12 Weeks	0.001	-0.776
Hamstring Isokinetic 60°/sec			
	Baseline	0.028	-0.521
	Preoperative	0.043	-0.475
	+ 6 Weeks	0.013	-0.591
	+ 12 Weeks	0.018	-0.565
Hamstring Isokinetic 120°/sec			
	Baseline	0.010	-0.609
	Preoperative	0.009	-0.620
	+ 6 Weeks	0.000	-0.893
	+ 12 Weeks	0.000	-0.798
Timed Chair-rise Test (TCT)			
Quadriceps Isokinetic 60°/sec			
	Preoperative	0.044	-0.473
Quadriceps MVIC			
	Preoperative	0.032	-0.508
Hamstring Isokinetic 120°/sec			
	Baseline	0.029	-0.519
	Preoperative	0.035	-0.497

p value – significance level; r value – correlation coefficient

Table 6.6. Correlations between uninvolved muscle strength and objective function

Comparison		p value	r value
Timed Walk Test (TWT)			
Quadriceps Isokinetic 60°/sec			
	Baseline	0.001	-0.767
	Preoperative	0.001	-0.763
	+ 6 Weeks	0.025	-0.534
	+ 12 Weeks	0.000	-0.789
Quadriceps Isokinetic 120°/sec			
	Baseline	0.003	-0.688
	Preoperative	0.002	-0.705
	+ 6 Weeks	0.004	-0.673
	+ 12 Weeks	0.000	-0.842
Quadriceps MVIC			
	Baseline	0.000	-0.798
	Preoperative	0.003	-0.701
	+ 6 Weeks	0.012	-0.596
	+ 12 Weeks	0.001	-0.749
Hamstring Isokinetic 60°/sec			
	Baseline	0.001	-0.776
	Preoperative	0.007	-0.642
	+ 12 Weeks	0.002	-0.710
Hamstring Isokinetic 120°/sec			
	Baseline	0.002	-0.714
	Preoperative	0.001	-0.739
	+ 6 Weeks	0.012	-0.596
	+ 12 Weeks	0.000	-0.798
Timed Stair-Climb Test (TST)			
Quadriceps Isokinetic 60°/sec			
	Baseline	0.000	-0.820
	Preoperative	0.000	-0.881
	+ 6 Weeks	0.013	-0.591
	+ 12 Weeks	0.003	-0.701
Quadriceps Isokinetic 120°/sec			
	Baseline	0.000	-0.824
	Preoperative	0.000	-0.820
	+ 6 Weeks	0.003	-0.693
	+ 12 Weeks	0.001	-0.749
Quadriceps MVIC			
	Baseline	0.000	-0.881
	Preoperative	0.000	-0.824
	+ 6 Weeks	0.008	-0.626
	+ 12 Weeks	0.003	-0.688
Hamstring Isokinetic 60°/sec			
	Baseline	0.000	-0.868
	Preoperative	0.004	-0.678
	+ 12 Weeks	0.008	-0.626
Hamstring Isokinetic 120°/sec			
	Baseline	0.000	-0.868
	Preoperative	0.001	-0.768
	+ 6 Weeks	0.008	-0.626
	+ 12 Weeks	0.001	-0.745
Timed Chair-rise Test (TCT)			
Quadriceps Isokinetic 60°/sec			
	Baseline	0.001	-0.735
	Preoperative	0.001	-0.771
Quadriceps Isokinetic 120°/sec			
	Baseline	0.002	-0.717
	Preoperative	0.004	-0.679
Quadriceps MVIC			
	Baseline	0.002	-0.713
	Preoperative	0.000	-0.829
Hamstring Isokinetic 60°/sec			
	Baseline	0.001	-0.759
Hamstring Isokinetic 120°/sec			
	Baseline	0.007	-0.642
	Preoperative	0.007	-0.634

p value – significance level; r value – correlation coefficient

Table 6.7. Correlations between subjective questionnaires and objective function

Comparison		p value	r value
WOMAC-Pain and TCT			
	Baseline	0.047	0.464
WOMAC -Function and TWT			
	Baseline	0.034	0.252
SF36-Physical Health and TWT			
	Baseline	0.009	-0.623
	+ 12 Weeks	0.041	-0.481
SF36-Physical Health and TST			
	Preoperative	0.039	-0.487
SF36-Mental Health and TCT			
	+ 12 Weeks	0.026	-0.528

p value – significance level; r value – correlation coefficient
WOMAC – Western Ontario McMaster Osteoarthritis Index
SF36 – Short Form 36
TWT = 25 metre walk test ; TST = Timed stair-climb test ; TCT = Timed chair-rise test

6.4 DISCUSSION

The main findings from the present study were that an 8 week quadriceps femoris neuromuscular electrical stimulation program significantly increased preoperative quadriceps and hamstring muscle strength of the involved limb in a cohort of patients undergoing TKA for end-stage OA, along with significant improvements in objective measures of functional capacity. These effects translated into earlier strength and functional recovery at 12 weeks postoperatively. The NMES group had less QFM atrophy than the control group at 12 weeks postoperatively when compared to baseline. Our compliance is higher than a recent study using NMES in subjects with knee OA (99% vs. 81%).(247) The device used in this study was garment-based which may have been more amenable to training, resulting in improved compliance.

At baseline, the involved limb of the NMES group was up to 30% weaker compared to the uninvolved limb. While both limbs increased in strength, presumably due to a cross-over effect in the uninvolved limb, the gains were greater in the involved limb so that preoperatively there was no significant difference in quadriceps or hamstring muscle strength between limbs. These improvements in the NMES group contrast with the active control group where it appears that simple advice on preoperative strengthening and range of motion exercises do not confer any preoperative benefit. This is further highlighted by changes in muscle mass where the NMES group increased QFM CSA by 7.4% while the control group decreased by 1.7%.

Using a similar home-based NMES program, Talbot et al found a 9% increase in QFM strength in patients with knee OA.(247) The larger increase in strength in the present study may be attributed to differences in training intensity. Their study provided NMES 3 days/wk for 12 weeks with stimulation intensity gradually increased. We encouraged our subjects to increase stimulation intensity to their maximal tolerated level during the initial 2 weeks. This was followed by a 6 week program at the highest tolerated intensity. The increase in

preoperative QFM strength compares favourably with previous studies evaluating exercise prehabilitation.(297;299;300) Since the preoperative improvements in strength occurred to a greater extent than QFM CSA, we speculate that the strength gains were primarily due to neurological adaptation resulting in increased muscle activation.(112)

The NMES prehabilitation program did not attenuate declines in postoperative muscle strength. Involved quadriceps and hamstring strength decreased by approximately 55%, indicating that similar mechanisms are responsible for early strength loss. This compares with previous reports that cited postoperative decreases in QFM strength of approximately 60%.(260;278) On the other hand, strength in the uninvolved limb did not decline in the postoperative period. Voluntary muscle activation failure, which is the main cause of strength loss in the early postoperative period, may be a unilateral phenomenon due to gamma loop dysfunction.(281;294) Activation failure has also been termed arthrogenic muscle inhibition (AMI) since it is generally accepted that painful stimulus from a diseased joint causes the reflex inhibition of muscle motor units.(151) However, Mizner et al found that knee pain was not a principle cause of activation failure following TKA.(302) Further work is clearly needed to elucidate the pathways potentiating muscle weakness after TKA.

Postoperative muscle atrophy, although not statistically significant, was more pronounced in the control group than the NMES group compared to baseline levels (12.1% vs.3.7%). A decrease in postoperative atrophy has also been reported by Rodgers et al (299) following exercise prehabilitation. Thus, improvements in pre-operative strength appear to attenuate muscle atrophy following TKA. Such a concept is supported by the strong correlations we found between muscle strength and CSA at all time-points.

Only the NMES group had significant preoperative improvement in the objective measures of functional capacity. This was most notable in the chair-rise test with an average improvement of 34%. At 6 weeks postoperatively both groups deteriorated by a similar amount in the time taken to perform both the stair-climb and 25-metre walk tests but not the

chair-rise test. When functional recovery was evaluated, significant improvements were only seen in the NMES group such that at week 12 postoperatively the NMES group was better in the performance of the stair-climb and chair-rise tests. It is interesting that significant between group differences were found with respect to function but not muscle strength preoperatively and postoperatively. Buchner et al described a curvilinear relationship between strength and functional capacity in frail elderly adults.(110) Small changes in strength were associated with substantial improvements in functional capacity. Similar findings have been reported in individuals with knee osteoarthritis.(283) Although not statistically significant, there may have been sufficient differences in muscle strength between the groups to produce the differences in functional capacity.

Strength recovery appears to be a common finding in prehabilitation studies, whereas improvements in functional capacity have only been described in a case report.(299-301) This paradox is difficult to explain, especially since we saw significant improvements in both strength and function in the NMES group. We also found associations between strength and i) TST at all time points, ii) TWT at baseline, week 5 and week 12 postoperatively and iii) TCT preoperatively. Differences in study designs may explain why other researchers did not find improvements in function. Rodgers et al (299) used a 10 metre timed walk test only to assess functional capacity, while Rooks et al (300) used a timed up and go test (TUG) which involves both chair-rise and 6 metre walk components. In our earlier study assessing the efficacy of NMES in subjects with knee OA (Chapter 4), we found that the timed stair-climb test was a more sensitive measure of function than the 25-metre walk test and also correlated more strongly with muscle strength than the chair-rise test. Given that we have seen similar patterns post-TKA in this study, we believe the timed stair-climb test should be considered the objective functional measure of choice when assessing efficacy of prehabilitation and rehabilitation programs in subjects with knee OA and after knee replacement surgery.

It was not possible to compare the preoperative effects of NMES on extension lag as it was only present in the NMES group at baseline. We cannot account for this difference since, both study groups had similar quadriceps strength at baseline. We found that declines in knee range of motion were greater in the control group than the NMES group (-19.8° vs. -7.2°) at 12 weeks compared to baseline. These changes were not statistically significant. Our small sample size may have limited the sensitivity of this measure. Minor et al., found no relation between knee ROM and patient perceived satisfaction.(259) In addition, preoperative ROM does not predict postoperative functional outcomes.(293) Such findings question the use of ROM as an outcome measure following TKA.

Overall, the self-report outcome scores did not reflect changes in the objective functional assessments. Surprisingly, preoperative SF-36-physical health scores improved in the control group despite them deteriorating in the objective measures of functional capacity. Nonetheless, the NMES group did report postoperative improvements in the Oxford and SF-36 scores as well as a reduction in pain by the WOMAC-pain subscale.

The benefits of knee arthroplasty itself may have masked smaller but significant improvements as a result of prehabilitation.(298;299) Expectations from surgery also influence patients' perception of function.(303-305) It is possible that subjective questionnaires are less sensitive than objective performance tests in measuring the effects of prehabilitation. We only found a moderate relation between the WOMAC function and the timed walk test. Several studies that determined no postoperative benefit with exercise prehabilitation in TKA only used subjective questionnaires to measure function.(297;298) Like us, they found limited improvements in outcome scores despite increases in muscle strength. Had they assessed function objectively they may actually have found beneficial effects of prehabilitation

It has been shown that exercise prehabilitation can shorten the length of hospital stay with a greater chance of going home on discharge rather than to a rehabilitation

facility.(297;300) The length of postoperative hospital stay was slightly lower in the NMES group in this study. In an era of cost-containment, any measure that can reduce the requirement for postoperative rehabilitation should be considered. A home-based preoperative muscle stimulation may be financially viable in TKA and a cost-analysis study is now warranted. Given the high compliance with this program, it could be utilised by patients independent of geographical location.

This study used a randomised control design, and all evaluations were performed with the assessor blinded to group assignment. Unlike previous studies, we employed several objective and subjective measures of function to determine postoperative recovery, as well as dynametric and radiological muscle evaluation. This global approach has permitted comparisons to be made with all prior studies of prehabilitation in total knee arthroplasty. The principle limitation of this study is the small sample size. This may have attributed to the lack of statistical power to detect changes in subjective outcomes and clinical measures.

In summary, we found that using NMES for 8 weeks preoperatively improves quadriceps strength and functional recovery following TKA. In addition, self-report outcome scores did not reflect changes in the objective functional assessments and may be considered less sensitive outcome measures in this setting. Given that no previous data exists on the use of NMES prehabilitation, the results from this study warrant further research on a larger TKA population as part of a cost-analysis study. We believe these results provide evidence for a role of preoperative NMES in expediting a return to normal activities in patients undergoing TKA for knee OA.

Chapter VII

CONCLUSIONS

The aims of this study were to investigate the effects of an 8 week neuromuscular electrical stimulation program on clinical parameters in subjects with end-stage knee osteoarthritis undergoing total knee arthroplasty and identify its effects on skeletal muscle at the molecular level.

We have shown that an 8 week unsupervised home-based NMES program can significantly improve preoperative performance in functional capacity in subjects with end-stage knee OA. This was associated with significant gains in muscle strength in the involved and uninvolved limbs (crossover effect) reflecting adaptations in neural signalling pathways. Subjects developed greater tolerance for higher stimulation intensities throughout the program, and we support the recommendation for conditioning periods when commencing NMES training. Although healthy subjects require electrically induced contraction force of at least 25% to increase muscle strength, subjects with knee OA can still obtain significant gains with lower values.

Increases in skeletal muscle strength appear to be mainly due to neurological adaptation as increases in strength were greater than muscle hypertrophy. We found evidence of a transition from a fast to slow MHC genotype in response to NMES. This is consistent with recent studies which reported similar patterns following exercise training. We saw significant upregulation of IGF-1 in response to NMES training. Effects on the muscle-specific E3 ubiquitin ligases (MURF-1 and MAFbx) were less dramatic.

The preoperative improvements in quadriceps strength and function translated into improved recovery at 12 weeks following TKA. Patient perceived outcome scores did not correlate strongly with performance in objective function and as such can be considered less sensitive outcome measures when evaluating the efficacy of prehabilitation.

This is the first study that has investigated the use of NMES in a cohort with end-stage disease. NMES may offer definitive therapy in subjects with who are unfit for or refuse TKA. We have shown that prehabilitation does improve recovery after TKA, and provided evidence on appropriate assessment modalities. NMES has several advantages over exercise programs as it is not limited by patient location and can be performed unsupervised with high levels of compliance. Further study is recommended on a larger population to confirm the results from this study.

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Appendix 1: Ethical Approval Letter

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14th June 2007 Our Ref: RW/03/2007/004

Mr. Raymond Walls,
109, Upper Leeson Street.
Dublin 4.

Re: Research Application
Effect of Preoperative Neuromuscular Electrical stimulation on Clinical Outcomes and Muscle Function following Total Knee Arthroplasty

Dear Mr. Walls,

I am writing to you in connection with the above and following a recent Ethics Committee meeting, I am pleased to inform you that this research project was approved at that meeting.

The Ethics Committee requested that a report be forwarded to them as to the outcome and success of this trial.

Yours sincerely,

Mr. Aidan Gleeson,
Chief Executive.
Direct line (01) 8140461
Email: aidan.gleeson@cappagh.ie

Information and Consent

The effect of Preoperative Neuromuscular Electrical Stimulation on the outcome of Total Knee Arthroplasty

This Informed Consent Form has two parts:
- **Information Sheet (to share information about the study with you)**
- **Certificate of Consent (for signatures if you agree to participate)**

PART I: Information Sheet

Introduction

This project is under the guidance of Professor John O'Byrne (Professor of Orthopaedics at the Royal College of Surgeons) in Cappagh National Orthopaedic Hospital and Professor Niall Moyna (Professor of Health and Human Performance) in Dublin City University. The research concerns the effect neuromuscular stimulation (NMES) prior to your total knee replacement (TKR) may have on the outcome of the operation. NMES is the application of electrical pulses through electrodes in a specially designed garment (KneeHAB) causing muscle contractions to increase muscle strength.

You are in no way obliged to partake in the study and if you do not wish to take part, it will in no way delay or affect your scheduled operation.
If at any stage there is anything that you do not understand, please stop and ask me to explain or alternatively you can ask me at the end.

Purpose

It is well known by clinicians that one of the main factors influencing the success of a total knee replacement is the strength of the quadriceps muscles – these are the muscles at the front of your thigh. By taking a little sample of your muscle tissue (a biopsy), we are planning to look for genes that may cause weakness in these muscles. In addition, by having NMES before your operation, we are looking to see if this affects the activity of any of these genes. The ultimate outcome will be whether your recovery from the operation has been positively affected.

Participant selection

You are being asked to participate as you are currently awaiting a knee replacement and satisfy the requirements of the study.

Voluntary Participation

Your decision to participate in this study is entirely voluntary. It is your choice whether to participate or not. If you choose not to consent, all the services you receive will continue and nothing will change. You may also choose to change your mind later and stop participating, even if you agreed earlier, and your operation will not be in jeopardy.

Permission will also have been sought from the Consultant looking after you before asking you to participate.

Procedures and Protocol

The main intervention involved will be 2 muscle biopsies: one 9 weeks before your operation and the second on the day of the operation when you have been anaesthetized. Biopsies are small samples of muscle that are taken through a tiny incision – smaller than your finger nail in length. They can easily be done under a local anaesthetic and you do not need to be put to sleep for it.
In addition, you will be assessed both clinically and with an MRI scan of your leg at these times and again 3 months following your operation. An MRI scan does not involve any ionizing radiation that could potentially be harmful to you. Part of the clinical assessment will include a test of muscle strength on a specially designed machine known as "Dynomometer".

Half of the patients in the study will be asked to use the stimulation garment (KneeHAB) to improve their muscle strength. The maximum stimulation intensity will be adapted to your own tolerance and will not involve severe discomfort. Some students from Dublin City University will be able to help you master this device and will call to your house to check your progress.

In order to see if there is any benefit in boosting your leg muscle strength pre-operatively, we need to make comparisons. Patients taking part in the study will be randomly placed into one of two groups. One group will receive the neuromuscular stimulation program and the other will continue on as normal until their operation. After the operation, both groups will receive the same postoperative management as is normal.

The muscle biopsies obtained during the study will be used only for the research outlined and will be destroyed once the research is complete.

Duration

Part of the design of the study is to minimize the inconvenience to you, the patient. Thus it is hoped that most of the necessary data can be collected and the first sample of muscle taken when you are attending the hospital for your routine Pre-operative workup. The second sample and assessments will be done while you are an inpatient for your operation. The final data collection will be around 3 months after your operation and would involve you attending the hospital for an hour or two. There will be 2 brief strength testing assessments before surgery and a further strength assessment 6 weeks after surgery, all in DCU.

Side Effects

With regard to the muscle biopsy, the side effects would be very minor and would include the risk of some bruising; some discomfort after the local anaesthetic wears off; and very rarely patients have reported a small temporary area of numbness. Local infection has also been occasionally reported.

Benefits

The ***POTENTIAL*** benefits to you may include a decrease in knee pain felt before surgery and during recovery as well as a more rapid post-operative recovery time.

The ***POTENTIAL*** benefits for the community would be that this may evolve into a program that is undertaken by all patients before their operation in order to improve the

outcome. Also, we may identify genes associated with muscle weakness that could be the target of a different type of therapy in the future.

Incentives

This study will not involve any monetary incentive to you. However, you will incur no costs for any tests performed and every effort will be made to minimize the inconvenience.

Confidentiality

The information that we collect from this research project will be kept confidential. Information about you that is collected from the research will be put away and no-one but the researchers will be able to see it.

Sharing of the results

The knowledge that we get from this study can be shared with you before it is made widely available to the public. Confidential information will not be shared. Afterwards, we will publish the results and present our findings at conferences in order that other interested people may learn from our research.

Right to Refuse or Withdraw

You do not have to agree to take part in this research if you do not wish to do so and refusing to participate will not affect your treatment in any way. You may stop participating in the research at any time that you wish without losing any of your rights as a patient here.

Who to Contact

If you have any questions you may ask them now or later, even after the study has started. If you wish to ask questions later, you may contact myself at the following address;
Research Investigator:

Dr. Raymond Walls
c/o Cappagh National Orthopaedic Hospital
Finglas, Dublin 11

Email: raywalls1@hotmail.com
Cappagh Telephone: 01-8140400
Emergency No: 087-296-3651

This proposal has been reviewed and ethical approval granted by the Health Research Board whose task it is to make sure that the research participants are protected from harm.

PART II: Certificate of Consent

I have been invited to participate in a study on Muscle Strength prior to my knee replacement. I understand that this will involve a total of 2 muscle biopsies (taking of muscle samples), several clinical assessments and scans, and possibly a neuromuscular stimulation program before my operation. I have been informed of the minimal risks involved.
I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate in this study and understand that I have the right to withdraw from the study at any time without in any way affecting my medical care.

Print Name of Participant: _____

Signature of Participant: _____

Date: ____/____/ 2007

I have accurately read or witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print Name of Researcher: _____

Signature of Researcher: _____

Date: ____/____/ 2007

A copy of this Informed Consent Form has been provided to the participant

_____**(initialed by researcher)**

Neuromuscular Electrical Stimulation Program 2007

Patient Logbook

SUBJECT NUMBER	

Pre-operative Total Knee Replacement Training

Thank you for participating in this study

Neuromuscular Electrical Stimulation Program - NMES

You have been assigned to the intervention group and will receive an 8 week long home-based neuromuscular electrical stimulation (NMES) program.

The portable, garment based stimulator (KneeHAB) will be applied to the quadriceps femoris muscle group of your arthritic knee; that is the knee which is due to be replaced by your orthopaedic surgeon in 8-9 weeks time.

It is proposed that quadriceps strength training will increase the strength of the quadriceps muscle resulting in improved function and decreased pain. It is believed it will bypass the mechanisms that precipitate muscle weakness associated with knee osteoarthritis.

The increase in quadriceps muscle strength may result in a more rapid recovery after your knee replacement surgery.

You will only be required to attend for 2 further strength testing assessments before your surgery; all other assessments will be performed when you attend for review as part of your normal pre-operative and post-operative hospital assessments.

A member of the research team will be in contact with you on a weekly basis to ensure you to discuss any questions that may arise during the program.

Should you experience any difficulty with the program at any stage please contact
Dr Raymond Walls in Cappagh Hospital (01-8140400); if there is an emergency call 087-2963651

Additional information is provided in the Information and Consent form already signed by you.

Study Schedule

Day 1:	Demonstration on using the portable stimulator
DCU and Cappagh scan	Full Clinical Assessment / Questionnaires / MRI
	Muscle Biopsy (Local Anaesthesia)
Week 1 (Baseline):	Further instruction on using the portable stimulator
DCU	Biopsy Wound Review Strength Testing

2 WEEK CONDITIONING PERIOD

Week 3:	Strength Testing
DCU	

6 WEEK TRAINING PERIOD

Week 6:	Strength Testing
DCU	
Week 9:	Full Clinical Assessment / Questionnaires / MRI scan
DCU and Cappagh	Strength Testing Muscle Biopsy (General / Spinal Anaesthesia on day of surgery)

KNEE REPLACEMENT SURGERY

Week 15:	Full Clinical Assessment / Questionnaires
DCU and Cappagh	Strength Testing
Week 21:	Full Clinical Assessment / Questionnaires / MRI scan
DCU and Cappagh	Strength Testing

STUDY COMPLETE

Using the Stimulator

TIME:

- Use the stimulator at the same time each **MORNING**
  - Between **8am and 10 am** ideally
- The sessions are programmed to last only **20 minutes**
  - Record the total duration used each time even if the session is cut short
  - You can pause the session and recommence it if you need to stand-up

POSITION:

- Remove the plastic liners over the electrodes
  - **Keep these safe** as they are needed to cover the electrodes after each session
- Put on the garment with the leg as straight as possible
- **THEN** attach the stimulator pack
- Perform the session **sitting on a chair with your feet on the ground with your toes against a wall**
- Have your knee bent to about **60 degrees**
  - Use the **cardboard cut-out** supplied to guide you in this position as previously shown

STIMULATION INTENSITY:

- Turn on the hand held stimulator
- Increase the stimulation until a **tolerable level** is reached using **both** dials
- You should try to **increase the intensity** during each 20 minute session
- You should try to increase the intensity at **each session**
- The device will **automatically stop** when the 20 minute session is complete

NB – PAUSE THE STIMULATOR (*ON/OFF Button*) BEFORE STANDING UP IF THE SESSION IS NOT COMPLETE

AFTER EACH SESSION:

- Check the **battery power** symbol
  - If only 1 power bar is remaining **RECHARGE** for 2-3 hours
- **Cover the electrodes** with the clear plastic liners
- **Fill in the Logbook:**
  - the maximum intensity achieved for both readings (0-99)
  - Total usage duration
- The device will turn off automatically should you forget

NMES Conditioning Period

Subject No:_____

- This initial 2 week period will involve neuromuscular training of the quadriceps femoris muscle of your arthritic knee providing 7 sessions in total.
- Sessions should be performed at the same time each morning. (**8 to 10 am**)
- Increase the stimulation intensity during each session
- If it is uncomfortable, lower the intensity and then gradually build it back up to a tolerable level
- Record the maximum level achieved for both sides of the muscle at the end of the session as well as the total duration of the session

Day	Date	Stimulation Intensity		Duration (Mins)
		Inside (VMO)	Outside	
Friday				
Sunday				
Tuesday				
Thursday				
Saturday				
Monday				
Wednesday				

NMES Training Period

Subject No:_____

- This is the main six week training program to increase your muscle strength
- Perform stimulation sessions the **same time each morning**
- Try to increase the stimulation during the program
- If it is uncomfortable, lower the intensity and then gradually build it back up to a tolerable level
- You will attend **Dublin City University** for strength testing at the end of **week 3** which is the mid-point of the training program.

Week 1

Day	Date	Stimulation Intensity		Duration (Mins)
		Inside (VMO)	Outside	
Friday				
Saturday				
Monday				
Tuesday				
Wednesday				

NMES Training Period

Subject No:_____

Week 2

Day	Date	Stimulation Intensity		Duration (Mins)
		Inside (VMO)	Outside	
Thursday				
Friday				
Saturday				
Monday				
Tuesday				

Week 3

Day	Date	Stimulation Intensity		Duration (Mins)
		Inside (VMO)	Outside	
Wednesday				
Thursday				
Friday				
Monday				
Tuesday				

NMES Training Period

Subject No:_____

Week 4

Day	Date	Stimulation Intensity		Duration (Mins)
		Inside (VMO)	Outside	
Wednesday				
Thursday				
Saturday				
Monday				
Tuesday				

Week 5

Day	Date	Stimulation Intensity		Duration (Mins)
		Inside (VMO)	Outside	
Wednesday				
Thursday				
Friday				
Saturday				
Monday				

NMES Training Period

Subject No:_____

Week 6

Day	Date	Stimulation Intensity		Duration (Mins)
		Inside (VMO)	Outside	
Tuesday				
Wednesday				
Thursday				
Friday				
Monday				

TRAINING PROGRAM COMPLETE

WELL DONE

Neuromuscular Electrical Stimulation Program 2007

Questionnaires

Subject Number	
Questionnaire Number	/4
Date	/ /

Pre-operative Total Knee Replacement Training

Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC)

Subject Number:_____ **Date**:_____

Please rate the activities in each category according to the following scale of difficulty;

0 = none; 1 = slight; 2 = moderate; 3 = very; 4 = extremely

Pain	Walking	
	Stair climbing	
	Nocturnal	
	Rest	
	Weight bearing	
Stiffness	Morning stiffness	
	Stiffness occurring later in the day	
Physical function	Descending stairs	
	Ascending stairs	
	Rising from sitting	
	Standing	
	Bending to floor	
	Walking on flat surface	
	Getting in/out of car	
	Going shopping	
	Putting on socks	
	Lying in bed	
	Taking off socks	
	Rising from bed	
	Getting in/out of bath	
	Sitting	
	Getting on/off toilet	
	Heavy domestic duties	
	Light domestic duties	
	Score (/96)	

SF 36 Patient Questionnaire

Subject Number: _____ **Date:**_____

Please answer every question. Some questions may look like others but each one is different.
Simply tick the box beside the answer that best describes your response.

1) In general, how would you say your health is?

- ☐ Excellent
- ☐ Very good
- ☐ Good
- ☐ Fair
- ☐ Poor

2) Compared to one year ago, how would you rate your health in general now?

- ☐ Much better than a year ago
- ☐ Somewhat better than a year ago
- ☐ About the same as a year ago
- ☐ Somewhat worse than a year ago
- ☐ Much worse than one year ago

3) The following items are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

	Yes, limited a lot	Yes, limited a little	No, not limited at all
a) Vigorous activities such as running, lifting heavy objects, participating in vigorous sports			
b) Moderate activities such as moving a table, pushing a vacuum cleaner, bowling or playing golf			
c) Lifting or carrying groceries			
d) Climbing several flights of stairs			
e) Climbing one flight of stairs			
f) Bending, kneeling or stooping			
g) Walking more than a mile			
h) Walking several blocks			

i) Walking one block			
j) Bathing or dressing yourself			

4) During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

a) Cut down the amount of time you spent on work or other activities

☐ Yes ☐ No

b) Accomplished less than you would like

☐ Yes ☐ No

c) Were limited in the kind of work or other activities

☐ Yes ☐ No

d) Had difficulty performing the work or other activities (for example it took extra time)

☐ Yes ☐ No

5) During the past 4 weeks have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

a) Cut down the amount of time you spent on work or other activities

☐ Yes ☐ No

b) Accomplished less than you would like

☐ Yes ☐ No

c) Didn't do work or other activities as carefully as usual

☐ Yes ☐ No

6) During the past 4 weeks to what extent has your physical health or emotional problems interfered with your normal activities with family, friends, neighbours or groups?

☐ Not at all
☐ Slightly
☐ Moderately
☐ Quite a bit
☐ Extremely

7) How much bodily pain have you had during the past 4 weeks?

☐ None at all
☐ Slight
☐ Moderate
☐ Quite a bit
☐ Extreme

8) During the past 4 weeks how much did pain interfere with your normal work (including both work outside the home and housework)?

☐ Not at all
☐ Slightly
☐ Moderately
☐ Quite a bit
☐ Extremely

9) These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one best answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks;

	All the time	Most of the time	A good bit of the time	Some of the time	A little bit of time	None
a) Did you feel full of pep?						
b) Have you been a very nervous person?						
c) Have you felt so down in the dumps nothing could cheer you up?						
d) Have you felt calm and peaceful?						
e) Did you have a lot of energy?						
f) Have you felt downhearted and blue?						
g) Did you feel worn out?						
Have you been a happy person?						
Did you feel tired?						

10) During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives etc.)?

- ☐ All of the time
- ☐ Most of the time
- ☐ Some of the time
- ☐ A little of the time
- ☐ None of the time

11) How TRUE or FALSE is each of the following statements for you?

	Definitely true	Mostly true	Don't know	Mostly false	Definitely false
a) I seem to get sick a little easier than other people					
b) I am as healthy as anyone I know					
c) I expect my health to get worse					
d) My health is excellent					

Oxford Knee Score

Subject Number:_____ **Date:**_____

Please circle the number beside the symptom which best answers each of the 12 questions

1) Pain	None	1	
	Very mild	2	
	Mild	3	
	Moderate	4	
	Severe	5	
2) Washing and drying self	No trouble	1	
	Very little trouble	2	
	Moderate trouble	3	
	Extreme difficulty	4	
	Impossible to do	5	
3) Getting into and out of car	No trouble	1	
	Very little trouble	2	
	Moderate trouble	3	
	Extreme difficulty	4	
	Impossible to do	5	
4) Walking time before pain becomes severe	Over 30 minutes	1	
	16 – 30 minutes	2	
	5 – 15 minutes	3	
	Around house only	4	
	Always severe walking	5	
5) Pain on Standing up after sitting	None	1	
	Mild	2	
	Moderate	3	
	Severe	4	
	Unbearable	5	
6) Limp due to knee	Rarely or never	1	
	Sometimes / just at first	2	
	Often – not just at first	3	
	Most of the time	4	
	All of the time	5	
7) Kneeling and getting up again	Easy	1	
	With little difficulty	2	
	With moderate difficulty	3	
	With extreme difficulty	4	
	Impossible	5	
Pain in bed at night	No nights	1	
	1 or 2 nights	2	
	Some nights	3	
	Most nights	4	
	Every night	5	
	Not at all	1	

9) Interference with housework due to knee pain	A little bit	2	
	Moderately	3	
	Greatly	4	
	Totally	5	
10) Feeling of giving way	Rarely / never	1	
	Sometimes / just at first	2	
	Often / not just at first	3	
	Most of the time	4	
	All of the time	5	
11) Shopping alone	Easy	1	
	Little difficult	2	
	Moderately difficult	3	
	Extremely dificult	4	
	Impossible	5	
12) Walking down a flight of stairs	Easy	1	
	Little difficult	2	
	Moderately difficult	3	
	Extremely dificult	4	
	Impossible	5	
		Total score (/60)	

Appendix 5: Supplementary Results Study 1

Quadriceps Femoris Neuromuscular Electrical Stimulation in Knee Osteoarthritis:

Effects on Muscle Strength and Clinical Function

Table A5.1. Isokinetic and Isometric Muscle Peak Torque

	Time			
	Baseline	**Week 2**	**Week 5**	**Week 8**
INVOLVED LIMB				
Isokinetic Quad 60°/sec (Nm)				
NMES	62.3 ± 33.3	70.2 ± 34.5 [a]	76.6 ± 42.1	82.8 ± 48.6 [ab]
Control	65.3 ± 20.0	76.8 ± 24.0	79.1 ± 23.6	76.5 ± 22.6
Isokinetic Quad 120°/sec (Nm)	58.2 ± 30.1	63.3 ± 34.5	65.0 ± 38.1	67.4 ± 43.6
NMES	57.4 ± 17.5	64.5 ± 21.8	66.9 ± 21.4	64.3 ± 20.0
Control				
MVIC Quad (Nm)				
NMES	87.4 ± 36.9	101.4 ± 45.4	106.0 ± 46.8 [a]	110.0 ± 56.1
Control	92.2 ± 31.2	103.1 ± 30.1	106.6 ± 30.8	105.5 ± 30.7
Isokinetic HS 60°/sec (Nm)				
NMES	39.9 ± 22.9	50.3 ± 27.1 [a]	55.0 ± 32.9	56.3 ± 32.5
Control	42.7 ± 13.1	48.7 ± 17.3	52.9 ± 12.6	55.7 ± 14.5
Isokinetic HS 120°/sec (Nm)				
NMES	38.1 ± 23.4	47.2 ± 26.7 [a]	51.3 ± 31.4	53.7 ± 29.9 [a]
Control	36.9 ± 10.7	44.5 ± 15.8	50.0 ± 17.6	46.2 ± 14.5
UNINVOLVED LIMB				
Isokinetic Quad 60°/sec (Nm)				
NMES	92.2 ± 48.7	93.5 ± 44.8	97.0 ± 49.1	101.0 ± 53.7 [a]
Control	68.9 ± 22.2	80.0 ± 16.0	81.4 ± 16.0	81.4 ± 19.3
Isokinetic Quad 120°/sec (Nm)	77.3 ± 40.9	77.3 ± 38.8	80.9 ± 41.6 [a]	83.0 ± 44.3
NMES	57.8 ± 19.2	65.6 ± 17.9	69.5 ± 16.5	69.1 ± 18.7
Control				
MVIC Quad (Nm)				
NMES	112.0 ± 54.5	119.2 ± 59.6	121.5 ± 58.3	130.6 ± 66.1
Control	95.2 ± 24.5	110.9 ± 23.2	111.6 ± 26.6	103.5 ± 25.1
Isokinetic HS 60°/sec (Nm)				
NMES	48.7 ± 42.2	53.8 ± 21.6	54.8 ± 26.1	59.4 ± 31.0
Control	45.0 ± 13.3	56.8 ± 11.6	56.0 ± 10.9	56.5 ± 14.8
Isokinetic HS 120°/sec (Nm)				
NMES	43.3 ± 22.8	46.2 ± 21.9	47.1 ± 25.9	53.4 ± 31.5
Control	39.7 ± 14.9	46.2 ± 10.9	44.9 ± 10.8	43.7 ± 9.1

Values are means ± SD;

Quad – Quadriceps; HS – Hamstring; MVIC – Maximum Voluntary Isometric Contraction.

[a] $p<0.05$ vs. baseline; [b] $p<0.05$ vs week 2

Table A5.2. Objective Functional Capacity

	Time	
	Baseline	**Week 8**
25m-Walk Test (secs)		
NMES	16.6 ± 5.4	15.2 ± 5.2 [a]
Control	18.3 ± 2.2	18.9 ± 4.8
Chair-Rise Test (secs)		
NMES	9.7 ± 3.9	6.6 ± 1.0 *[a]
Control	9.1 ± 1.1	8.4 ± 0.9
Stair-Climb Test (secs)		
NMES	17.6 ± 11.0	14.2 ± 9.4 [a]
Control	18.4 ± 5.8	18.6 ± 6.8

Values are means ± SD; [a] $p<0.01$ vs. baseline; *$p < 0.05$ vs. control

Table A5.3. Self-Report Outcome Measures

	Time	
	Baseline	**Week 8**
WOMAC Pain		
NMES	11.3 ± 2.8	11.2 ± 3.2
Control	9.7 ± 5.2	11.3 ± 3.7
WOMAC Function		
NMES	37.2 ± 10.7	34.0 ± 10.6
Control	38.0 ± 14.3	38.3 ± 12.8
WOMAC Stiffness		
NMES	4.9 ± 2.8	5.1 ± 1.7
Control	5.0 ± 1.5	5.8 ± 1.6
SF-36 Physical Health		
NMES	45.0 ± 24.4	48.7 ± 22.2
Control	38.2 ± 18.7	45.3 ± 16.0 [a]
SF-36 Mental Health		
NMES	66.8 ± 19.9	65.7 ± 19.6
Control	59.8 ± 21.9	69.5 ± 20.7 [a]
Oxford		
NMES	35.5 ± 8.0	35.0 ± 7.3
Control	35.7 ± 8.8	35.2 ± 6.5

Values are means ± SD; [a] $p<0.05$ vs. Baseline
WOMAC – Western Ontario McMaster Osteoarthritis Index;
SF-36-Short Form36; Oxford – Oxford Knee Score

Appendix 6: Supplementary Results Study 2

Quadriceps Femoris Neuromuscular Electrical Stimulation in Knee Osteoarthritis:

The Mechanisms Associated with Strength Gain

Table A6.1. Isokinetic and Isometric Muscle Strength

	Time			
	Involved Limb		**Uninvolved Limb**	
	Baseline	**Week 8**	**Baseline**	**Week 8**
Isokinetic QFM 60°/sec (Nm)				
NMES	55.4 ± 26.6 *	74.9 ± 44.3 [a]	85.8 ± 47.0	91.5 ± 47.2 [a]
Control	65.3 ± 20.0	76.5 ± 22.6 [a]	68.9 ± 22.2	81.4 ± 19.3 [a]
Isokinetic QFM 120°/sec (Nm)				
NMES	51.7 ± 23.2	58.7 ± 35.9	70.4 ± 36.7	74.3 ± 36.8
Control	57.4 ± 17.5	64.3 ± 20.0 [a]	57.8 ± 19.2	69.1 ± 18.7 [a]
MVIC QFM (Nm)				
NMES	78.9 ± 26.7	95.4 ± 33.7 [a]	101.5 ± 45.7	117.8 ± 55.4 [a]
Control	92.2 ± 31.1	105.5 ± 30.7 [a]	95.2 ± 24.5	103.5 ± 25.1 [a]

Values are means ± SD; [a] $p<0.05$ vs. baseline; * $p<0.05$ vs Uninvolved limb

QFM – Quadriceps; HSM – Hamstring; MVIC – Maximum Voluntary Isometric Contraction.

Table A6.2. Quadriceps Femoris Muscle Cross-Sectional Area

	Time	
	Baseline	**Week 8**
Involved CSA (cm^2)		
NMES	39.8 ± 11.2	42.8 ± 11.9 [a]
Control	42.1 ± 7.0	41.6 ± 7.5
Uninvolved CSA (cm^2)		
NMES	45.0 ± 13.7	45.2 ± 13.5
Control	44.6 ± 10.2	44.3 ± 9.7

Values are means ± SD; <0.05 vs. Baseline

CSA – cross-sectional area

Table A6.3. Expression of Genes Associated with Muscle Anabolism and Catabolism

	Time	
	Baseline	**Week 8**
IGF-1(AU)		
NMES	0.79 ± 0.43	1.41 ± 1.08
Control	0.51 ± 0.12	0.73 ± 0.13[a]
MAFbx (AU)		
NMES	1.28 ± 0.40	1.10 ± 0.42
Control	1.04 ± 0.34	1.01 ± 0.33
MURF-1 (AU)		
NMES	0.94 ± 0.34	0.87 ± 0.21
Control	0.92 ± 0.32	0.85 ± 0.24

Values are means ± SD; [a] <0.05 vs. Baseline

Table A6.4. Myosin Heavy Chain Isoform Gene Expression

	Time	
	Baseline	**Week 8**
MHC-1(AU)		
NMES	1.23 ± 0.53	1.10 ± 0.76
Control	0.84 ± 0.48	1.05 ± 0.61
MHC-IIa (AU)		
NMES	1.21 ± 0.31	1.28 ± 0.72
Control	0.95 ± 0.63	0.94 ± 0.63
MHC-IIx (AU)		
NMES	1.16 ± 1.10	0.67 ± 0.72
Control	1.28 ± 0.83	1.47 ± 1.03

Values are means ± SD

AU: Arbitrary Units (expression to relative GAPDH)

Appendix 7: Supplementary Results Study 3

Efficacy of a Prehabilitation Program in Total Knee Arthroplasty using Neuromuscular Electrical Stimulation

Table A7.1. Isokinetic and Isometric Muscle Strength

	Time			
	Baseline	**Preoperative**	**+ 6 Weeks**	**+ 12 Weeks**
INVOLVED				
Isokinetic Quad 60°/sec (Nm)				
NMES	63.3 ± 35.1	86.1 ± 50.3 [a]	39.2 ± 19.8 [b]	52.2 ± 28.1 [b]
Control	64.3 ± 22.2	76.6 ± 25.3	33.8 ± 18.2	44.1 ± 23.3
Isokinetic Quad 120°/sec (Nm)				
NMES	59.1 ± 31.7	69.5 ± 45.7	35.5 ± 15.6 [b]	46.9 ± 21.0
Control	55.5 ± 18.9	62.6 ± 21.9	29.9 ± 17.3	38.9 ± 17.9
MVIC Quadriceps (Nm)				
NMES	87.4 ± 39.2	111.7 ± 59.3	53.8 ± 17.2 [b]	80.9 ± 35.7 [c]
Control	89.5 ± 34.1	100.3 ± 31.3	47.4 ± 23.4	61.7 ± 26.5
Isokinetic HS 60°/sec (Nm)				
NMES	36.6 ± 21.7	54.5 ± 33.9 [a]	26.2 ± 21.5	35.0 ± 18.9
Control	41.2 ± 14.0	52.4 ± 13.5	22.2 ± 8.3	27.8 ± 13.5
Isokinetic HS 120°/sec (Nm)				
NMES	34.9 ± 22.4	51.2 ± 30.5 [a]	30.4 ± 24.6	34.4 ± 17.9
Control	36.0 ± 11.7	44.0 ± 15.1	18.2 ± 10.5	25.9 ± 10.6
INVOLVED				
Isokinetic Quad 60°/sec (Nm)				
NMES	90.3 ± 51.3	99.7 ± 56.8 [a]	95.4 ± 54.2	97.7 ± 57.4
Control	61.6 ± 14.8	76.2 ± 16.4	79.1 ± 17.5	75.8 ± 18.8
Isokinetic Quad 120°/sec (Nm)				
NMES	75.8 ± 43.1	80.4 ± 46.1	75.3 ± 43.8	80.3 ± 46.5
Control	51.5 ± 12.5	64.4 ± 16.4	62.2 ± 14.6	60.8 ± 14.2
MVIC Quadriceps (Nm)				
NMES	108.4 ± 56.5	128.0 ± 69.6	119.8 ± 60.5	128.2 ± 66.5
Control	86.8 ± 15.0	95.6 ± 17.7	100.4 ± 18.1	100.7 ± 15.5
Isokinetic HS 60°/sec (Nm)				
NMES	45.6 ± 23.4	55.6 ± 30.3	55.1 ± 25.6	56.7 ± 24.7
Control	41.1 ± 10.6	51.6 ± 9.5	53.0 ± 5.7	50.0 ± 6.9
Isokinetic HS 120°/sec (Nm)				
NMES	40.6 ± 22.5	42.8 ± 25.2	44.9 ± 18.9	49.6 ± 25.1
Control	35.9 ± 13.2	41.6 ± 8.4	42.3 ± 8.3	37.9 ± 6.9

Values are means ± SD; [a] $p<0.05$ vs. baseline; [b] $p<0.05$ vs. preoperative; [c] $p<0.05$ vs. + 6 Weeks;
Quad – Quadriceps; HS – Hamstring; MVIC – Maximum Voluntary Isometric Contraction.
+6 Weeks - 6 weeks post TKA; + 12 Weeks – 12 Weeks-Post TKA

Table A7.2. Quadriceps femoris muscle cross-sectional area

	Time		
	Baseline	**Preoperative**	**+ 12 Weeks**
Involved QFM CSA (cm^2)			
NMES	39.1 ± 11.7	41.9 ± 12.4	37.6 ± 9.7 [a]
Control	39.8 ± 4.7	39.1 ± 4.9	35.0 ± 2.9
Uninvolved QFM CSA (cm^2)			
NMES	42.5 ± 12.3	42.8 ± 12.2	42.1 ± 12.1
Control	41.7 ± 8.2	41.3 ± 7.2	40.4 ± 6.5

Values are means ± SD; [a] $p<0.05$ vs. Preoperative
CSA – Cross-sectional Area of the quadriceps muscle

Table A7.3. Objective Functional Capacity

	Time			
	Baseline	**Preoperative**	**+ 6 Weeks**	**+ 12 Weeks**
25m-Walk Test (secs)				
NMES	17.3 ± 5.2	15.7 ± 5.1 [a]	21.3 ± 6.1 [b]	16.4 ± 5.0 [c]
Control	18.9 ± 1.8	19.9 ± 4.5	25.0 ± 7.7	20.3 ± 3.5
Chair-Rise Test (secs)				
NMES	10.1 ± 3.9	6.6 ± 1.1 *[a]	7.7 ± 1.4	6.4 ± 1.1 *[c]
Control	9.2 ± 1.2	8.4 ± 1.0	8.4 ± 1.7	8.3 ± 1.3
Stair-Climb Test (secs)				
NMES	18.7 ± 11.2	15.0 ± 9.6 [a]	25.0 ± 10.9 [b]	16.0 ± 7.1 *[c]
Control	19.9 ± 5.1	20.7 ± 4.9	36.1 ± 15.6	25.9 ± 8.7

Values are means ± SD; +6 Weeks - 6 weeks post TKA; + 12 Weeks – 12 Weeks-Post TKA
[a] $p<0.01$ vs. baseline; [b] $p<0.01$ vs. preoperative; [c] $p<0.05$ vs. + 6 Weeks;
*$p < 0.05$ vs. control